STUDY ON SERUMCHOLINESTERASELEVEL INDEATHS DUE TO ORGANOPHOSPHORUS COMPOUNDS POISONING

Dissertation submitted in partial
fulfillment of the requirements for the
degree

M.D.(Forensic Medicine)

BRANCH - XIV

INSTITUTEOFFORENSICMEDICINE

MADRAS MEDICAL COLLEGE

CHENNAI–600003

THE TAMILNADU

Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL2016

BONAFIDECERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"STUDY ON SERUMCHOLINESTERASE LEVEL IN DEATHS DUE TO ORGANOPHOSPHORUS COMPOUNDS POISONING"** has been carried out by **Dr. S.KARTHIGA DEVI** a Post Graduate student under my supervision and guidance for his study leading to Branch-XIV M.D. Degree in Forensic Medicine during the period of November 2014to September 2015

DEAN
Madras Medical College&
RajivGandhiGovt.General Hospital
Chennai 600003

Date:
Place:

Director andProfessor
Institute of ForensicMedicineMadras
Medical College
Chennai 600003

Date:
Place:

DECLARATION

I **Dr. S.Karthiga Devi** solemnly declare that this dissertation titled **"STUDY ON SERUM CHOLINESTERASE LEVEL IN DEATHS DUE TO ORGANOPHOSPHORUS COMPOUNDS POISONING"** is the bonafideworkdoneby me under the expert guidance and supervision of **Prof. Dr. R.Vallinayagam**, Director and Professor, Institute of Forensic Medicine, Madras Medical College, Chennai – 3. This dissertation is submitted to The TamilNaduDr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D. Degree (Branch-XIV) in Forensic Medicine.

Place: **Dr.S.Karthiga Devi**

Date:

ACKNOWLEDGEMENT

I am greatly obliged to the Dean, **Dr.Vimala,** MD, Madras Medical College and Rajiv Gandhi Govt. General hospital, Chennai-3forallowing me to conduct this study.

I am especially thankful to my Director and Professor **Dr.R.Vallinayagam, MD.**, Associate Professor **Dr.M.N.RajamaniBheemRao, M,D.,**and Associate Professor **Dr.T.Vedanayagam, MD.,** Institute of Forensic Medicine, Madras Medical College, Chennai-3 for their interest and encouragement, in bringing out thisdissertation for my MD exam.

I am thankful to my Assistant Professor **Dr.S.Ramalingam, MD.,** and Assistant Professor **Dr.R.Narendar, M.D.,** Institute of Forensic Medicine,Madras Medical college, Chennai-3 for their help and encouragement in bringing out this dissertation.

I am thankful to **Dr.K.Ramadevi, M.D.,** Director and Professor, **Dr. V.Amudhavalli, M.D.,** Professor, Institute of Biochemistry, Madras Medical college, Chennai-3. I thank all the technicians of institute of Biochemistry for their help in doing this study.

I thank all my Colleagues for helping, in collecting material for my study.

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. Karthiga Devi
Postgraduate M.D.(Forensic Medicine)
Madras Medical College
Chennai – 600 003.

Dear Dr. Karthiga Devi,

The Institutional Ethics Committee has considered your request and approved your study titled **"Study on the serum cholinesterase level in deaths due to organophosphorus compounds poisoning". No.11112014.**

The following members of Ethics Committee were present in the meeting held on 11.11.2014 conducted at Madras Medical College, Chennai-3.

1. Dr.C.Rajendran, M.D.,	: Chairperson
2. Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3. Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4. Prof.R.Nandini, M.D., Inst.of Pharmacology, MMC	: Member
5. Prof.P.Ragumani, M.S., Professor, Inst.of Surgery, MMC	: Member
6. Prof.Md.Ali, M.D., D.M., Prof. & HOD of Medl.G.E., MMC	: Member
7. Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
8. Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
9. Prof.S.G.Sivachidambaram, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
10. Thiru S.Rameshkumar, Administrative Officer	: Lay Person
11. Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
12. Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

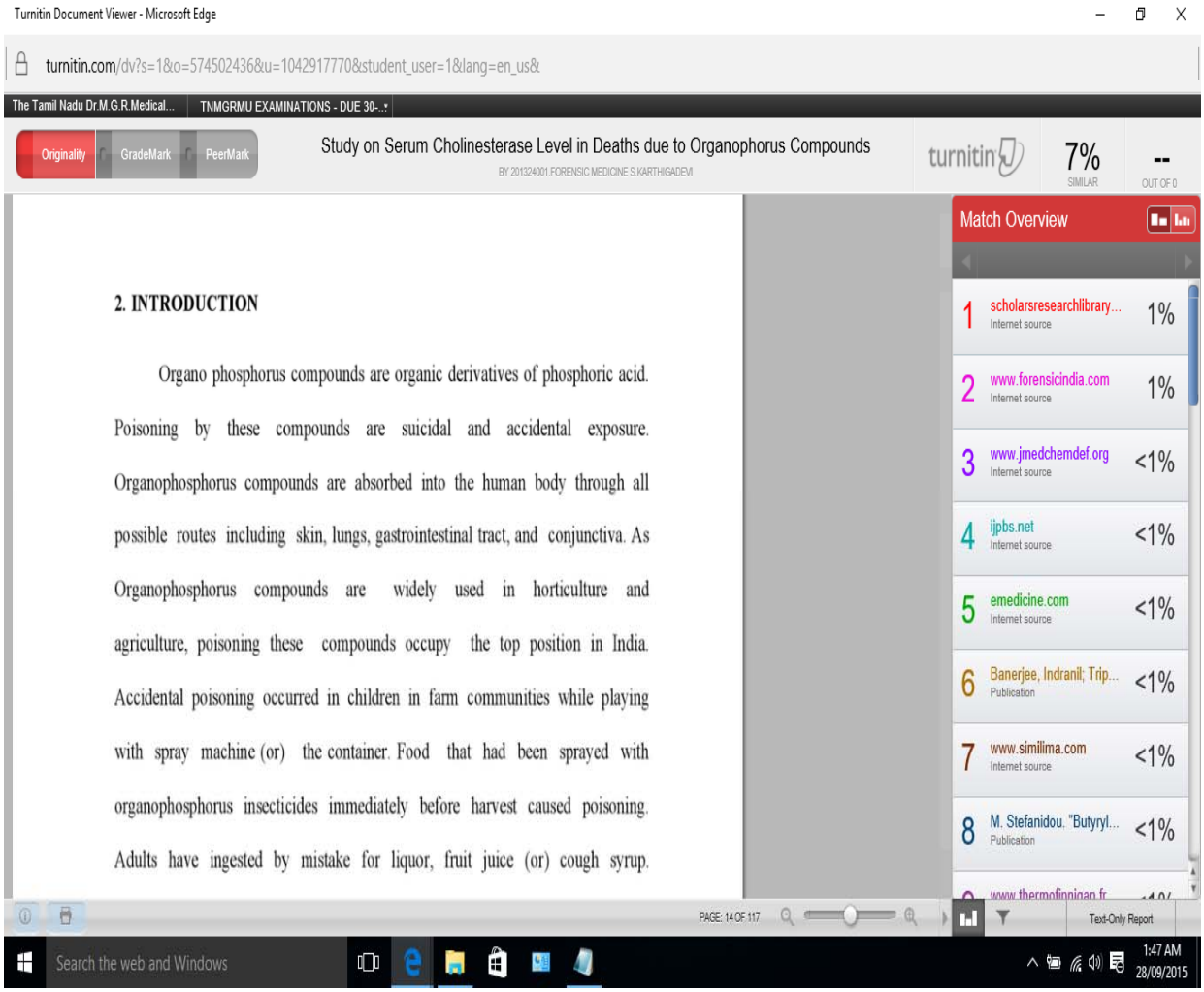
We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

25/11/14

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

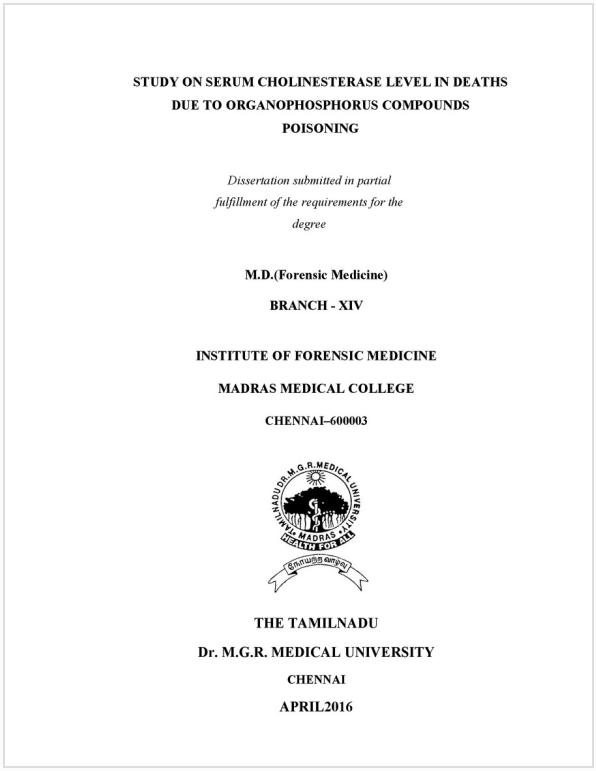

Turnitin Document Viewer - Microsoft Edge
turnitin.com/dv?s=1&o=574502436&u=1042917770&student_user=1&lang=en_us&
The Tamil Nadu Dr.M.G.R.Medical...
TNMGRMU EXAMINATIONS - DUE 30-..
Originality
GradeMark
PeerMark
Study on Serum Cholinesterase Level in Deaths due to Organophorus Compounds
BY 20132400 1.FORENSIC MEDICINE S.KARTHIGADEVI
turnitin
7%
SIMILAR
--
OUT OF 0
2. INTRODUCTION
Organo phosphorus compounds are organic derivatives of phosphoric acid. Poisoning by these compounds are suicidal and accidental exposure. Organophosphorus compounds are absorbed into the human body through all possible routes including skin, lungs, gastrointestinal tract, and conjunctiva. As Organophosphorus compounds are widely used in horticulture and agriculture, poisoning these compounds occupy the top position in India. Accidental poisoning occurred in children in farm communities while playing with spray machine (or) the container. Food that had been sprayed with organophosphorus insecticides immediately before harvest caused poisoning. Adults have ingested by mistake for liquor, fruit juice (or) cough syrup.
Match Overview
1 scholarsresearchlibrary... Internet source 1%
2 www.forensicindia.com Internet source 1%
3 www.jmedchemdef.org Internet source <1%
4 ijpbs.net Internet source <1%
5 emedicine.com Internet source <1%
6 Banerjee, Indranil; Trip... Publication <1%
7 www.similima.com Internet source <1%
8 M. Stefanidou. "Butyryl... Publication <1%
PAGE: 14 OF 117
Text-Only Report
Search the web and Windows
1:47 AM
28/09/2015

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	201324001.forensic Medicine S.kar…
Assignment title:	TNMGRMU EXAMINATIONS
Submission title:	Study on Serum Cholinesterase Le…
File name:	SerumlevelThesis_By_SKarthigade…
File size:	1.41M
Page count:	117
Word count:	17,077
Character count:	101,918
Submission date:	28-Sep-2015 01:29AM
Submission ID:	574502436



STUDY ON SERUM CHOLINESTERASE LEVEL IN DEATHS
DUE TO ORGANOPHOSPHORUS COMPOUNDS
POISONING

Dissertation submitted in partial
fulfillment of the requirements for the
degree

M.D.(Forensic Medicine)

BRANCH - XIV

INSTITUTE OF FORENSIC MEDICINE

MADRAS MEDICAL COLLEGE

CHENNAI–600003

THE TAMILNADU

Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL2016

ABBREVIATIONS

I.P.C	-	Indian Penal Code
Cr.P.C	-	Criminal Procedure Code
OPC	-	Organophosphorus Compounds
HETP	-	Hexa Ethyl Tetra Phosphate
TEPP	-	Tetra Ethyl Tetra Phosphate
OMPA	-	Octa Methyl Tetra Phosphate
ECG	-	Electro Cardio Gram
CPK	-	Creatine Phospho Kinase
LDH	-	Lactate Dehydrogenase
IgG	-	Gamma Immunoglobulin
IgM	-	Mu Immunoglobulin
TNF	-	Tumour Necrosis Factor
RBC	-	Red Blood Corpuscle
GCS	-	Glasgow Coma Scale
SCHE	-	Serum Cholinesterase
WHO	-	World Health Organisation

INDEX

LIST OF FIGURES

ABSTRACT

1. ABSTRACT

Organophosphorus compounds are widely used in agriculture and horticulture. Poisoning by these organo phosphorus compounds are accidental exposure or with suicidal intention.

Self consumption of organophosphorus compounds is a common worldwide health problem..These compounds are absorbed through skin, conjunctiva, lungs, and gastro intestinal tract. Immediately after absorption these compounds inhibit the enzyme acetyl cholinesterase leading to accumulation of acetylcholine producing morbidity and mortality. Pralidoxime and atropine are the drugs of choice in the treatment of OP poisoning.

The aim of the study is to estimate the serum cholinesterase levels in deaths due to OP poisoning.

1.1 Method of study: Prospective study.

36 subjects who died from OP poisoning, in the Rajiv Gandhi government general hospital, subjected for autopsy, to the Institute of Forensic Medicine, Madras Medical College, were selected. 28 were males and 8were females.

Serum cholinesterase level was done at the Institute of Biochemistry, Madras medical college, Chennai. The mean serum cholinesterase level was 1675+/-1228U/L.(Normal range . 6971-11900U/L).

Though there are various comorbid conditions that predict the morbidity and mortality in organophosphorus poisoning , the serum cholinesterase estimation is of diagnostic value in Forensic Practice.

Key Words:

Organophosphorus compounds, serum cholinesterase, acetyl choline, atropine, pralidoxime

INTRODUCTION

2. INTRODUCTION

Organo phosphorus compounds are organic derivatives of phosphoric acid. Poisoning by these compounds are suicidal and accidental exposure. Organophosphorus compounds are absorbed into the human body through all possible routes including skin, lungs, gastrointestinal tract, and conjunctiva. As Organophosphorus compounds are widely used in horticulture and agriculture, poisoning these compounds occupy the top position in India. Accidental poisoning occurred in children in farm communities while playing with spray machine (or) the container. Food that had been sprayed with organophosphorus insecticides immediately before harvest caused poisoning. Adults have ingested by mistake for liquor, fruit juice(or) cough syrup. Commonest organophosphorus compounds used for poisoning are parathion, malathion, monocrotophos, and dimethoate[1,2].

The Organophosphorus compounds irreversibly bind to cholinesterase causing phosphorylation and deactivation of acetyl cholinesterase resulting in accumulation of acetyl choline at the neural synapse causing an initial overstimulation followed by exhaustion and disruption of post synaptic neural transmission in the neural system. If the organophosphorus cholinesterase bond is not broken by pharmacologic intervention within 24 hours, large amounts of cholinesterase are destroyed causing morbidity and death. Normal level of cholinesterase:5300-10,000units/l.

The diagnosis of cause of death in forensic practice is based on the history given, post-mortem findings, viscera analysis report, and histopathology report. Failure to detect poison in viscera analysis is due to vomiting after consumption, lower dosage, stomach wash, antidote administration, poison absorption and elimination. The analytical techniques are more dependable and satisfactory.

The serum cholinesterase estimation is diagnostic of organophosphorus compounds poisoning. There is no decrease in activity in samples up to three weeks after death. To provide prophylaxis for occupational exposure. Protective clothing to cover head, neck, gloves, boots, and eye shields. Agricultural spray restricted to2-4hours per day not more than 6 days in a week[1,2].

To encourage Early hospitalization if symptoms appear. Serial cholinesterase estimation and administration of antidotes during clinical management.

AIMS AND OBJECTIVES

3. AIMS ANDOBJECTIVES

3.1 OBJECTIVE(S) / AIM:

3.1.1 Primary Objective: To **Study on Serum Cholinesterase Level in deaths due to Organophosphorus compounds Poisoning.**

3.1.2 Secondary Objective: To provide an evidence based report of the cause of death in deaths due to organophosphorus compounds poisoning. To determine the correlation between serum cholinesterase level and deaths due to organophosphorus compounds poisoning.

3.2.The majority of the deaths in poisoning cases are due to organophosphorus compounds as it is easily available for agricultural purposes.

3.3.Organophosphorus compounds are irreversible inhibitors of the enzyme cholinesterase.

3.4.The enzyme depression occurs within few minutes to hours after consumption of organophosphorus compounds.

3.5 Depression of serum cholinesterase enzyme is the biochemical indicator of excessive absorption of organophosphorus compounds.

3.6 .Normal serum cholinesterase level is 5300-10,000 units/l. Levels below 870units/l indicate poor prognosis and mortality.

3.7 To estimate the cut off levels of serum cholinesterase level at which mortality is inevitable.

REVIEW
OF
LITERATURE

4. REVIEW OF LITERATURE

4.1 Poison: Poison is defined as any substance when introduced into the living body or brought into contact with produces ill-health or death by its local or systemic effects[1].

A substance which is harmless in small quantities may act as a poison in large amount.

Toxicity is the ability to produce harmful effects. Lethal dose is the dose that is fatal to a healthy person.

Toxicity rating is done by gosselin :

Fatal dose schedule:

Less than five mg/kg is super toxic.

Five mg-Fifty mg/kg is extremely toxic.

Fifty mg-Five hundred mg/kg is very toxic.

Five hundred mg –Five gm/kg is is moderately toxic.

Five gm –Fifteen gm/kg is slightly toxic.

More than fifteengm/kg is non toxic[1].

4.2 Laws of Poisons in India:

Sec.39, Sec.40 Cr.Pc, Sec.176 I.P.C-Doctors treating the cases of homicidal poisoning should report to the Police.

Sec.193 I.P.C- Punishment for furnishing false information about poisoning cases.

Sec.175 cr.P.C.,Sec.201 I.P.C,Sec.202 I.P.C,-Doctors should inform about consumption of poison by the patients and the treatment given by the doctor to the police.

Sec.284 I.P.C- negligence in handling poisonous substances.

Sec.299 I.P.C-Culpable homicide including poisoning .

Sec.300I.P.C-Murder including by a method of poisoning.

Sec.304A I.P.C- Homicide by rash and negligent act including poisoning.

Sec.324 I.P.C-Causing hurt by dangerous weapons including poisoning.

Sec.326 I.P.C-Causing grievous hurt by dangerous weapons including poisoning.

Sec.328 I.P.C –Causing hurt by intentional poisoning.

The poison act 1919 amended in the year 1958 and in 1960 regulates launching, licensing, importation and sale of poisons in India[2].

4.3 Epidemiology of Poisoning:

Epidemiologyis the study , distribution, and determinants of the health related states and events in the population and control of the health problems.It refers to the field of medicine concerned with determination of the cause, incidence, and characteristic behaviorof the disease affecting the human populations.

It also includes the inter relationships of the agent, host, and environment as related factors for the distribution and control of the disease.The mode of poisoning is Suicidal, homicidal,exhibitional, (to create sympathy) accidentalto kill animalsand to kill insects. The suicide victim prefers to end his life with less agony[2].

An ideal suicide poison is easily available, cheap, quick in action,easily soluble in food or drinks, and producing painless death. Some examples are opium, barbituratesand organophosphorus compounds[1].

An ideal homicidal poison is should be

(i) without any obvious colour, taste or any specific smell.

(ii)available easily

(iii)capable of being mixed with food or drink.

(iv)difficult to be diagnosed clinically, with slow signs and symptoms.

(v)very toxic.

(vi)The metabolism and excretion of the homicidal poison should be rapid and mimic natural disease.

(vii)should not have any anti dote.

(viii)should not have post mortem changes and capable of being detected by any methods. Some examples are arsenic,aconiteand thallium[1].

The incidence of poisoning is three million cases worldwide with two lakhs and twenty thousand deaths each year.Commonest choice of suicide among people is to consume poison. Among thepoison consumption, 67% was pesticide as it is easily available. In India it is not accurate as most cases are not reported and estimated to that about 5-6 persons die due to poisoning [4].

In India pesticides and insecticides are widely used by farmers to increase the productivity of crops, fruits, and vegetables. Farmers do not follow the recommended schedule inspraying the insecticides. Accidental poisoning as occupational exposure is common while spraying insecticides[4].

Among the fruits and vegetables, pomegranate, grapes, cabbage, tomato, cauliflower etc. are sprayed with pesticides[4].

Mass disaster due to accidental food contamination also occurs in India.Kerala food poisoning tragedy: This miserable incidence happened in1958. Wheat flour and sugar were stored in the same cabin of ship which contained parathion an OP compound.Due to leakage they mixed together. Following the

consumption of the contaminated product , resulted in deaths of more than hundred people[11].

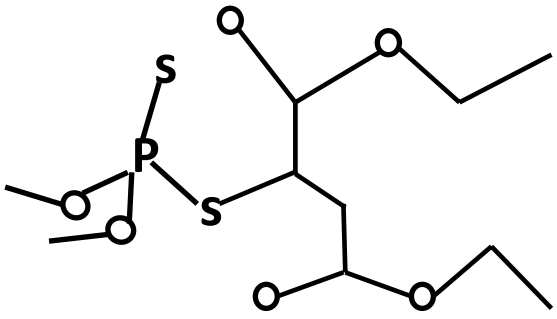
Bihar mid-day meal tragedy on 17th July 2013 suffered from OPC poisoning after eating mid-day meal in a school. 22 children died. This was due to storage of a cooking oil in an OPC container[11].

Homicide by OPC is not possible because of the kerosene like odour.

Suicidal poisoning by OPC is common as it is easily available.

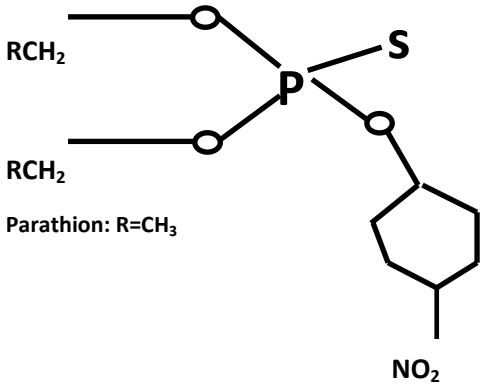
Organophosphorus compounds are esters of phosphoric acid. OPCs are under the classification ofagricultural poisons[1].

Malathion



12

Parathion



4.4 Classification Organophosphorus compounds**:**

4.4.1. Alkyl phosphates:

(i)HETP(Hexa ethyl tetra phosphate),

(ii)TEPP(Tetra ethyl tetra phosphate),

(iii)malathion(kill bug),

(iv)OMPA(octa methyl tetra phosphate)[1].

4.4.2.Arylphosphates:

(i)Parathion(follidol,Killphos),

(ii)Paraxon,

(iii)Methyl parathion,

(iv)Chlothion,

(v)Diazinon(Tik20)[1].

These compounds are available as dusts, granules, and liquids. These compounds are mixed with aromax a solvent which imparts a kerosene like smell[1,10].

Pictures of Organophosphorus compounds used as insecticides

4.5 History:

Phillipe de Clermont reported the synthesis of TEPP an organo phosphorus compound at a meeting of the French academy of sciences in 1854[10].

Lange in the year 1932 recognised the toxicity of the OPC that inhalation of the vapoursproducing choking sensation and dimness of vision[10].

Highly toxicOPCs such as sarin were used as warfare.

TEPP was the first OPC synthesized in the year 1854.

4.6 Uses oforganophosphorus compounds:

(i)In agriculture they are used as aerial spray mixed with liquid, or applied directly into the soil. When sprayed absorption occurs in leaves and stems .When applied intothe soil absorption occurs through roots.

When the insect sits on the plant, poison is absorbed through the exoskeleton, or when it eats the leaves of the plant it takes the poison orally[4].

(ii)Malathion is used for killing adult mosquitoes to prevent the spread of dengue fever and mosquito borne encephalitis[15].

(iii)Abate(Temephos) is used for control of anopheles stephensi mosquitoes in wells[15].

(iv) Fenthion is used as spray at the rate of 100mg/sqft to control the larvae of culexfatigans mosquitoes [15].

4.7. Absorption of OPC:

Plants absorborganophosphorus compounds through leaves and stems[4].

Picture of a person engaged in insecticide spraying

In human beings, absorption occurs through the skin, lungs, stomach, and mucous membranes.The rate of absorption of OPC depends upon the permeability of the clothing, contact time with the skin, nature of the skin, presence of injury on the skin, and total area of exposure. Finer powders are rapidly and completely absorbed. Parathion is absorbed more easily from the scrotal skin, skin of the axillae, skin of the head and neck than from skin of the hands and arms[16].

4.7.1 Metabolism and accumulation: Detoxification is bycytochromeP450 monooxygenase. After absorption OPCsaccumulate in liver, kidney, and salivary glands.The metabolites are excreted mostly through kidneys. Lesser amounts are excreted through faces and expired air[1].OPCs crosses the placenta and produces harmful effects on the foetus.OPC crosses the blood brain barrier[16].

4.8 Actions:

OPCs are inhibitors of the enzyme acetylcholinesterase and pseudocholinesterase. Acetylcholine is a neurotransmitter at the synapses.It is hydrolysed to choline and acetic acid by cholinesterasein the plasma. OPCs bind to the esteric site of the enzyme and inactivate it byphosphorylation. Hence they are called as cholinesterase inhibitors.Due to the accumulation of large amounts of unhydrolysed acetyl choline ,results in a syndrome of overactivity with paralysis of nerve and muscle. This condition becomes permanent in one to two days[1].

4.8.1 Latent Poisoning:

Signs and symptoms are absent. Diagnosis is based on serum cholinesterase level. Usually notreatment is required. Observation forsix hoursto watch forclinical manifestations. Mild poisoning occurs when cholinesterase activity is twenty to fifty percent of the normal level.Signs and symptoms are cramps, head ache, sweating, salivation, tightness in the chest. The prognosis is good. Moderate poisoning occurs when cholinesterase activity is ten to twenty percent of the normal level. Miosis,tremors,fasciculations,confusion and unableto walk. Prognosis is good with treatment.Severe poisoning occurs when the cholinesterase activity is less than ten percent of the normallevel.Miosis, cyanosis,respiratory insuffiency, seizures, secretions from mouth and nose.Paralysis and coma.Prognosis:Fatal without treatment[11].

4.9 Symptoms:

Muscarinic: Bronchoconstriction,Salivation,Lachrymation,Micturition,defaecation,vomiting,constriction of pupils,dimness of vision.

Nicotinic: fasciculation of muscle, cramps, absence ofreflexes.

CNS manifestations: Head ache, tremors, coma, convulsions,depression of the respiratory and cardiovascular centre[2,3].

4.9.1 Intermediate syndrome:

This occurs 24-96 hoursafter consumption. Muscle weakness, paralysis,acute respiratory paresis due to cholinesterase inhibition and muscle necrosis.Delayed peripheral neuropathy: This occurs 7-28 days after exposure characterized by ataxia, weakness and toe drop[2,3].

4.9.2 Toxicological effects on the body:

Respiratory system: Progressive bradypnoea leading to apnoea due to depression of the respiratory centre.

Cardiovascular disorders: OPC induced myocardial necrosis was reported by Povoa et al. Increase in creatinine kinase, and lactate dehydrogenase was reported by saadeh et al.other manifestations are sinus tachycardia, sinus bradycardia, hypertension, hypotension, and impaired force of contraction.ECG changes are prolonged QTC interval, ST segment elevation, extra systoles, and prolonged PR interval.

Central nervous system : The complications following chronic or acute OPC poisoning include impairment of memory, parkinsonian and pseudo bulbar signs, psychiatric and neuro psychological dysfunction and cerebellar syndrome[16].

Liver: serum bilirubin is increased .Jaundice, liver enlargement, increased urine urobilinogen abnormal liver function tests were also reported.(Maruyama,1954)congestion, centilobularnecrosisand fatty changes.

Hormonal imbalance: several experimental studies revealed that there is hormonal imbalance of sex hormones and developmental anomalies following pesticide exposure[16].

Oesophageal changes: oesophago-gastroscopy revealed circumferential hyperemia, and bleeding[16].

Renal effects: studies done by Ontario college showed that there is a positive association between solid tumours and pesticide exposure.It was reported that chronic exposure to pesticides resulted in renal failure[16].

Oxidative stress: OPC poisoning is associated with increased lipid per oxidation, reduced glutathione levels, and increased oxidative stress[16].

Among the organophosphorus compounds,parathion and malathion are commonly used.Parathion is most toxic, and commonly used poison . Even 0.1 mg/kg produced a sensation of uneasiness, warmth, tightness of the abdomenand frequent urination. The reduction in plasma cholinesterase level was 3% in one subject and 5% in another subject without any symptom. The oral fatal doseis estimated to be 1.43mg/kg[18].

Malathion is one of the least toxic organophosphorus insecticide. Even ingestion of 16mg of malathion for47 days continuously by volunteers did not alter serum cholinesterase levels or cause any symptoms. But 24mg ingestionfor 56 days caused 25% reduction in cholinesterase levels. Dermal application of 1%,5%,or 10% malathion 5times weekly for 8-16 weeks did not cause any symptoms or change in cholinesterase levels. Exposure of air containing 0.15gm,0.6gm or,2.4gm of malathion per 1000ft 84 times in 42 continious days did not cause any symptoms or change in cholinesterase levels. Death due to malathion ingestion occurred at 5gm, 25gm, 35gm, 70gm, and 60-90gms[18].

The Fatal dose:

(i) Highly toxic:OMPA(200mg),TEPP(100mg),Parathion(100mg).

(ii) Slightly toxic:malathion(1gm), Diazinon(1gm)

(iii) Fatal period: Within 24 hours in untreated cases.

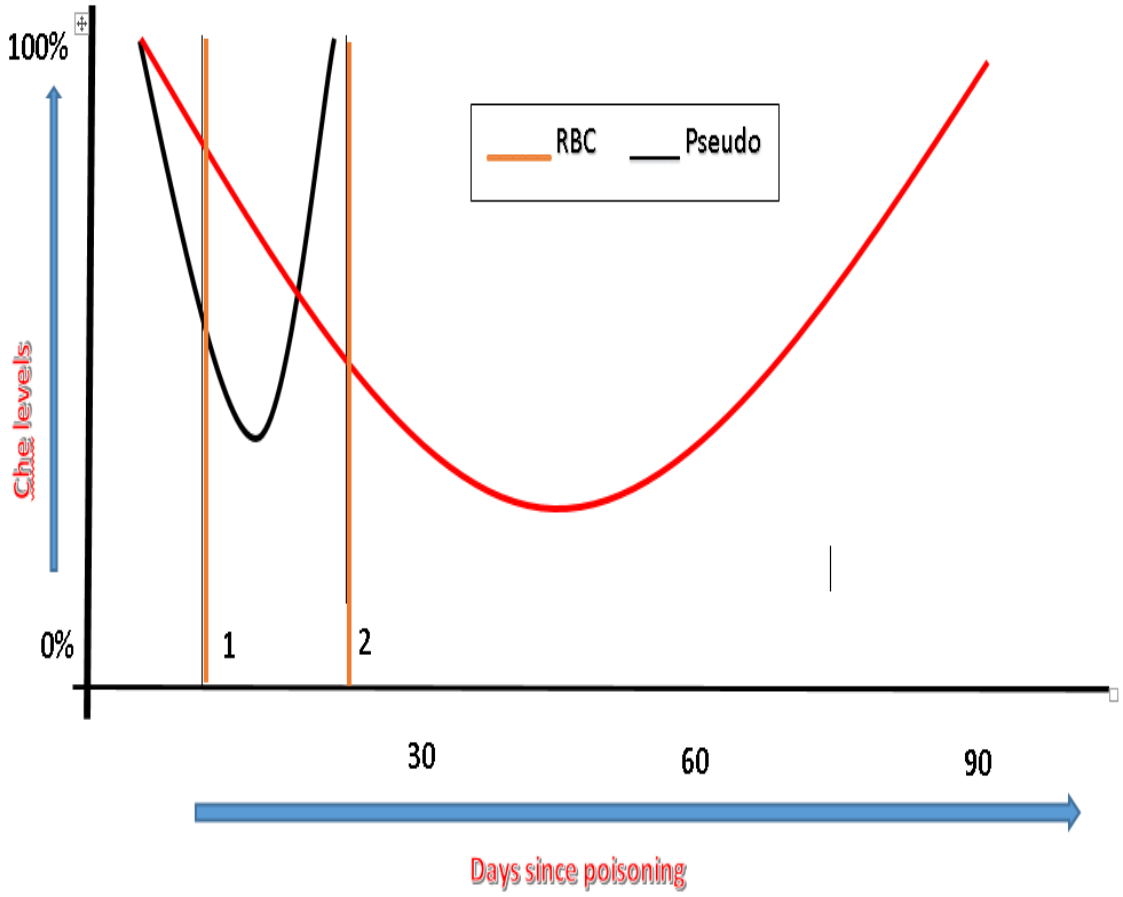
(iv) 10 days in cases when the treatment is not successful.

(v) Causes of death: Paralysis of respiratory muscles.

(vi) Broncho constriction.

(vii) Ventricular arrhythmias.

Diagnosis:(i)serum cholinesterase level normal (5300-10,000 units/L) reduction in both RBC and plasma cholinesterase level. Plasma cholinesterase level falls quickly and a better indicator of OPC poisoning[2].



Picture: Graphical representation of Interpretation of RBC &Pseudocholinesterase levels[11].

True cholinesterase is present in red cells, nervous tissue, and skeletal muscles. Pseudo cholinesterase is present in plasma, liver,heart, pancreasand brain. The value reaches normal level in four weeks[3].

The demonstration of cholinesterase at the motor end plate of the muscles can be done upto several months if the tissues are stored at four to six degree centigrade and at room temperature for one to two days. Cholinesterase activity can be demonstrated even in embalmed bodies[3].

(ii)Injection of 2mg of atropine causes atropinisation in a normal person and in OPC poisoned persons the symptoms are relieved[3].

(iii)Chemical test: P-nitro phenol excreted in cases of parathion poisoning can be demonstrated by addition of sodium hydroxide to the steam distillate of urine which imparts a yellow colour[11].

(iv)Blood investigations: Neutrophil Leucocytosisobserved in 46% of patients[16].

(v)Electrolyte changes: Hypokalemia[16].

(vi)Other biochemical changes: Hyperglycemia, increase in serum amylase[16].

(vii) Urine Investigations: Glycosuria, proteinuria[16].

(viii)ECG changes: Sinus bradycardia, atrioventricular block, ST, T wave changes, QTC prolongation may be observed[16].

Treatment OPC Poisoning :

(i) OPC poisoning is a medical emergency. Airway is maintainedand oxygen is administered. Patient is placed in left lateral position to avoid the risk of aspiration and this also reduces absorption of poison by decreasing gastric emptying.

(ii)The poison exposed areas of the patient are washed with soap and water.Supportive therapy is givenby administration of IV fluids[7].

(iii)Stomach wash done with 1 in 5000 potassium permanganate solution[7].

(iv)Activated charcoal 1gm/kg to be given. Activated charcoal is a mechanical antidote, which neutralizes the poison by mechanical action or prevent its absorption.It is a black coloured tasteless powder without any smell. It is prepared from various organic chemicals, by treating them at high temperatures with activating agents such as steam, carbon dioxide to increase its adsorptive capacity. The particles are small and the surface area is large. It ismixed with 8ml of water per gram of charcoal[7,11].

A cathartic such as sorbitol or magnesium citrate can be given if there is no diarrhoea.Drugs likephysostigmine, endrophoniumchloride andsuccinyl choline should be avoided[7].

(v)Atropine givento control muscarinic actions and CNS effects. It is not effective to control nicotinic actions.It has no effect on the respiratory centre in the presence of asphyxia. It is given in the dose of 2-4mg i.v (0.05mg/kg in children) and may be repeated every 10-15 min. until the tracheobronchial tree is cleared of the secretions and muscarinic symptoms are relieved. Signs ofatropinisation are dilated pupils, dry skinand skin flushing[7].

Average requirement is 40mg/day. Regular auscultation of the lungs should be done to assess the quantity of secretions. Atropine also acts on the brain by reducing the use of glucose and prevents the development of convulsions. It should not be given to a cyanotic patient for the risk of inducing ventricular fibrillation. Can be given after correcting cyanosis. Should not be given if heart rate exceeds 140/min.Glycopyrronium bromide is another

muscarinic antagonist like atropine with fewer side effectsbut adequate studies have not been done[7].

(vi)Oxygen in adequate quantities.

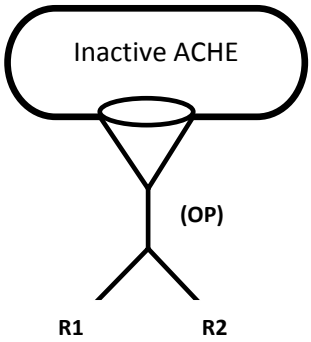
(vii) Specific cholinesterase reactivators like(a) (DAM) diacetylmonoxime or(b) 2-PAM (Pralidoxime iodide) to compete with OPC for the phosphate group and release it from cholinesterase enzyme.Actions of the oximes include reactivation of the inhibited cholinesterase and prevention of formation of phosphorylated AchE, which OPCs cannot bind further.Oximesalso directly detoxify theorganophosphorus compounds. Lethal concentrations of acetylcholine were detected in animal brains which were given pralidoximethere by saving from death due to organophosphorus compounds[7].

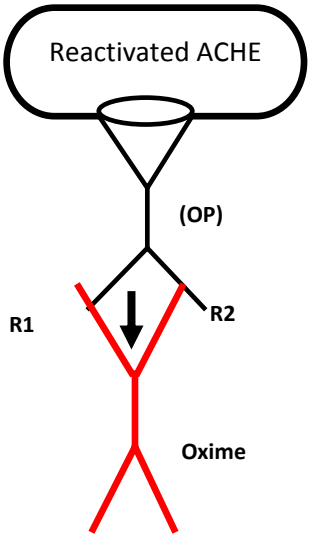
This indicates that reactivation alone is not the action of oximes.Dephosphorylation becomes difficult after some hours. (Milosevic,1969,1970).Pralidoxime was discovered byWilson andcolleguesin 1950. It has got 4 salts:mesilate,metisulfate, iodide and chloride.Iodide and chloride salts are commonly used. France, Belgium and UK use mesilateandmetisulfate[18].

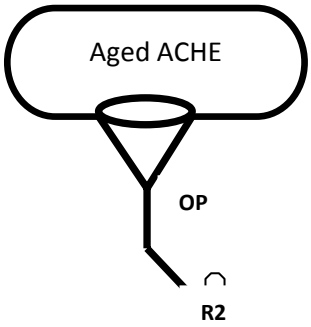
As chloride has smaller molecular weight of 173 than 264 of Iodide and provides 1-5 times active compound per gram of salt it is preferred over iodide. Moreover, a high dose of pralidoxime iodide puts the patient a risk of thyroid toxicity. The effectiveness of oximes in OPC poisoning in human beings was first demonstrated by Namba (1956) and Hiraki(1958)[18].

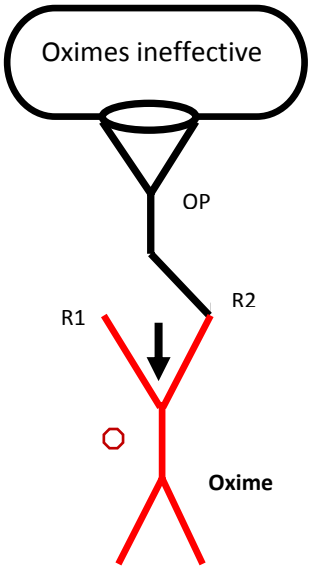
When volunteers were given iv injections of 15-20 mg pralidoxime iodide they experienced dizziness, blurred vision, diplopia,tachycardia ,head ache impaired accommodation, nausea , lasting for several minutes.In another study no ill effects occurred following i.m or iv injections of pralidoxime chloride 15-20mg/kg. Ingestion of 1-10gm of pralidoxime iodide by volunteers experienced tension,bitter taste, fatigue in the jaw, and rhinitis, thirty minutes after ingestion till two hours. When pralidoxime was given orally in a single dose of 7gm it produced no ill effects[18].

Mode of inhibition of Cholinesterase by OPC and reactivation by Oximes









The maximumdoseofoximes should not exceed 12gm one day. As it acts on the nicotinic sites it improves the muscle strength. But should cautiously used as rapid i.v injection produces temporary worsening of cholinergic manifestations as oximes are mildly anticholinergic.They bind to cholinesterase before regenerating it.This also causes tachycardia, diastolic hypertension,vomiting. Hagedornoximes: Hagedorn and coworkers synthesized this newer oximes which are effective against OP nerve gases and all known OPCs. NAAK (Nerve Agent Antidote Kit) is a dual chamber auto injector containing atropine 2mg, and 2-pralidoxime chloride 600mg which can be used against OPC war gases such as Tabun, sarin, and soman[7].

(vii)Magnesium sulphate is used to block the ligand gated calcium channels which results in decreased acetylcholine release and reduced CNS activity[16].

(ix) Convulsions controlled with diazepam. It is given in the dose of 5-10mg slow i.v.upto 30mg in adults.

(x)Antibiotics to prevent infections.

(xi)Exchange transfusiongives dramatic effect.

(xii)Cholinesterase administration from human plasma, erythrocytes or from animals is not effective as cholinesterase activity at the synapses are not affected[12].

(xiii)As the toxicity of the OP compounds is mediated by generation of nitric oxide and free radicals addition of vitaminC, VitaminE, melatonin, and low molecular weight thiols can be given to counteract their effects[12].

(xiv)Brazil and Iran use sodium bicarbonate as an alternative to oximes as increase in pH is found to improve the outcome in OPC poisoning[12].

(xv)The military search now aims at injection of cloned butylcholinesterase enzyme before exposure to OPnerve gases. But such a prophylactic approach is not practical for self-poisoning cases[12].

(xvi) Use of recombinant bacterial phosphodiesterase or hydrolases may break down OPC pesticides enzymatically and protect from poisoning. As it reduces the concentrations of OPC pesticides it allows the optimum activity of other treatments[12].

(xvii)Future drugs:Huperzine A produced from the extracts of huperziaserrata, a new drug used in the symptomatic treatment of alzheimer's disease in china is proved to be powerful inhibitor of acetyl cholinesterase. It penetrates through the blood brain barrier, has high oral bioavailability, longer duration of Ache inhibitory action, minimal cholinergic side effects and no unexpected toxicity[12].

(xviii)ZT-1 is a new dug with similar properties as that of HupA, and a promising drug of the chinese scientists to move into the international pharmaceutical markets[12].

Prophylaxis:

(i)Protective clothing.

(ii)Two hours spraying per day not more than six days continuously.

(iii)Spraying machines and containers to be washed and properly maintained.

(iv)If symptoms appear immediate medical help to be sought.

Due to the toxic nature of pesticides and the high risk involved in treatments, there should be on preventing pesticide illness rather than relying on treatment.Indoor use of OP compounds based pesticides have to be avoided, they are designed to be used outdoors only.Always read and follow the pesticides label's instructions and safety warnings all times.

Possibly use ready-to-use products without any mixing

All foodstuffs and drinkable items from the vicinity of pesticides and better keep them sufficiently covered.

4.10 Diagnosis OPC poisoning:

(i)Collection of the inquest report.

(ii)Post mortem appearances :

(a)Signs of asphyxia are noted.

(b)Congestion of the face.

(c)cyanosis of the lips and fingers.

(d)Smell of kerosene like odour in the stomach contents.

(e)Soft and flabby heart.

(f)Congested lungs with sub-pleural petechial hemorrhages.

(g)Brain congested and oedematous[11].

(iii)**Viscera preservation and chemical analysis:**

Stomach with its contents.

Upper thirty centimeters of small intestinewith its contents.

200gms of liver.

One half of each kidney.

100gms of brain.

Thirty ml of blood.

Thirty ml of urine should be sent with saturated sodium chloride solution as preservative[19].

As poisons are usually consumed orally, it is most likely to be present in the stomach and intestinal contents and their walls. After absorption they enter liver which is a detoxicating organ.The kidney is the organ of excretion and that also contains large quantities of poison that is excreted in the urine. Solid viscera should not be contaminated with gastrointestinal contents since the time of consumption of the poison can be assessed from the stomach contents. Poison in the stomach but not in the solid viscera gives the suspicion about the proof of absorption.Therefore the alimentary contents should be preserved separately.Poison in the urine is an evidence of absorption and excretion unless added purposely to create false evidence.The stomach contents can estimate the quantity of poison ingested[6].

The containers should be glass bottles with thousand ml capacity,wide mouthed and provided with glass stoppers.Rubber inserts can extract poison and should be avoided.All containers should be cleaned with sulphuric acid-chromate solution, rinsed with distilled water and dried.

Preservatives : saturated solution of sodium chloride to be used as a preservative .

While dispatching the viscera stomach and its contents in one bottle, intestine and its contents in one bottle.liver and kidney in another bottle, 100gm of brain in another bottle. Only $2/3^{rds}$ of the bottle should be filled with viscera and the preservative as the gases of decomposition may cause bursting of the bottle. The quantity of the viscera and the preservative should be equal. The bottles should be labelled with the name of the victim, age, autopsy number, police station, crime number, organ it contains date and the place of autopsy preservative used and signature.The stoppers should be covered with a piece of cloth tied by string and sealed .The sealed bottles are put into a box locked along with by department seal. Copies of inquest report and authorisation from magistrate are sent to the forensic science laboratory along with the viscera.

Cause of death in poisoning cases is based on evaluation of clinical, autopsy, toxicologic and circumstantial evidence[6] .

Fate of poisons in the body: The large amount poison is eliminated from the body as a result of vomiting and through defecation. The portion

absorbed is deposited in the liver in a less soluble form which is either metabolized or destroyed. The unaltered portion enters the general circulation. If the poison is not destroyed, it acts on the whole body or the target organ[1].

The absorbed portion is excreted by the kidneys and to some extent by the skin.Other routes of excretion of poison are bile, milk, saliva, mucous and serous secretions[1].

Local action poison: The local action is action produced by coming in contact the part. Congestion and inflammation by the irritant poisons chemical destruction by corrosives.Remote action is produced by either shock acting reflexly through severe pain caused by corrosives .The action of the poison produced after absorption in the blood and exerting specific action on the organs and tissues.Combined actions is having both local and systemic effects[1].

Causes modifying the action of poisons:Larger the quantity of poison consumed , severe were the toxic effects.A large quantity consumed orally may cause vomiting resulting in elimination and decreased toxicity. Some poisons may be toxic at a lower dosage. Poisons in a gaseous state act rapidly than poisons in a liquid state or solid state[1].

Mechanical combination: The action of poison is altered if combined mechanically with inert substances. Small dose of mineral acid produces corrosive action but when diluted with large amount of water it is harmless[1].

Absorption : Absorption from the stomach more rapid when empty , than it is full. Absorption is rapid when the stomach contents dissolves the poison.In gastro enterostomy poison enters the small bowel rapidly. Metabolic action of the liver makes some poisons inactive[1].

Condition of the body: Poisons have a greater effect on both extremes of age. A child below 2years of age does not have drug metabolizing enzymes , and blood brain barrier and more affected by the drugs[7].

Idiosyncrasy: It is an inherent personal hyper sensitivity to the agent in question. Habbit:The effect of certain poisons decrease with habituationTolerance is the ability of the organism to less response to a specific dose of a chemical than it was for the same dose[7].

The state of health: A diseased person will not tolerate the effects of the drugs as that of a healthy person. The action is delayed if a person is in an intoxicated condition or goes to sleep.Poisons which are eliminated slowly accumulate in the body and produce its effects[7].

4.11 Types of poisoning:

(i) Acute poisoning is caused by excessive single dose or several small doses over a short period of time.

(ii) chronic poisoning is caused by smaller doses over a period of timeresulting in gradual worsening.

(iii)**Sub acute poisoning:** shows features of both acute and chronic poisoning.

(iv)Fulminant poisoning: is due to ingestion of a massive dose producing death suddenly.

Parasuicide: voluntary manipulative act done to get rid of an intolerable situation.

Suspicion of poisoning:

i)sudden onset of symptoms in a healthy person.

ii)symptoms appearing after food or drink.

iii)increasing severity of the symptoms.

iv)Detection of poison in the food or excretory products is confirmatory.

Failure to detect poison in viscera analysis may be due to vomiting of the poison, diarrhoea, metabolism of the poison in the body, insufficient material for analysis, excretion of the poison from the body, treatment and administration of antidotes[20].

Estimation of serum cholinesterase level. other causes of reduced serum cholinesterase level are liver disorders, neoplasia, malnutrition, infection, drugs (morphine, succinylcholine), carbamate poisoning.

Serum levels of OPCs have got limited importance as OPCs are active at very low levels and varies with genetic differences among the individuals[21].

Chronic poisoning: This is seen in persons involved in spraying insecticide regularly due to inhalation and skin contamination. They suffer from weakness of muscles, muscle cramps, paraesthesias, ataxia, confusion, and psychiatric disorders[21].

Histopathology: Kidneys: epithelial necrosis. Liver: Liver is resistant as it is able to produce serum cholinesterase . Swelling of the cells of the liver parenchyma with glycogen depletion may occur.Heart: Perivascular hemorrhages of the myocardium may occur. Brain: Perivascular hemorrhages may occur. Skeletal muscle: muscle necrosis may occur[11].

Laurence lepage, Franscoiseschlele, Rene gueguen and Gerard siest studied about the other factors involved in cholinesterase activity. Genetic status, weight,(subscapular skinfold)increase in levels seen in overweight persons, hormonal status(post-menopausal women had a higher level)., use of oral contraceptives reduced the levels of serum cholinesterase[21].

Males 4-15 years-9930 u/l,15-25years9430u/l,25-55years9770u/l,>55years 9710u/l at 97.5 centiles.

Females 4-15 years -9850u/l, 10-25 years-8840u/l,25-55years8760u/l,45-55years-10665u/l,>55 years-10045u/l.(21)

Estimation of time since death is an objective of an autopsy.studies have been undertaken by researchers to find out postmortem interval by analyzing the serum levels of various enzymes.The available literature on biochemical (enzymal) changes in the post mortem blood (serum) is contributed to forensic scientist from temperate countries.Serum enzyme levels after death: No significant change in cholinesterase levels in samples analysed periodically upto three weeks after death was reported by Petty et al [22] .

Cholinesterase: An enzyme that acts as a catalyst in the hydrolysis of acetyl choline to choline and acetic acid.This enzyme is present through out the body, but is particularly essential at the neuro muscular junctions where the nerve fibres terminate.when a nerve impulse reaches the neuromuscular junction , acetylcholine is released. It then diffuses across the synaptic cleft and binds to the cholinergic receptors on the muscle fibres causing them to contract.cholinesterse splits the acetyl choline into components and stops the stimulation of the muscle fibres.The end products are taken by the nerve fibres and resynthesised to acetyl choline[30].

Cholinesterase inhibitor:Drugs like physostigmine, pyridostigmine. These are drugs of choice in myasthenia gravis., a disease which affects cholinergic receptors by auto antibodies. These drugs extent the effect the effect of acetyl choline in the muscle fibre. Cholinesterase reactivators are the enzymes used in treating OP poisoning[30] .

Acetylcholine is an ester of acetic acid and choline.Henry hellet dale identified acetyl choline in 1915 for its action on the heart tissue.Acetylcholine transmission in cardiac tissue lowers the heart rate.In skeletal muscle and neuromuscular junctions it is excitatory.Acetylcholine has functions in central and peripheral nervous system[31].

Acetylcholine stimulates the skeletal muscle in the peripheral nervous system. In the autonomic nervous system,it is a neuro transmitter.Acetylcholine binds to the receptors on the skeletal muscles, by opening the ligand gated sodium

channels in the cell membrane.As sodium enters the muscle cell, it initiates a series of steps to produce muscle contraction. Though it produces contraction of the skeletal muscle, it produces inhibitionof the cardiac muscle fibres [31] .

Acetylcholine is synthesizedfrom choline and acetyl coA by the action of the enzyme choline acetyl transferase in the neurons.The enzyme acetylcholinesterase splits acetylcholine into acetic acid and choline.Neurotoxins act by inhibiting acetylcholinesterase causing accumulation of acetylcholine at the neuromuscular junction leading to paralysis of muscles of respiration [31] .

DR.Bharatkumar, Dr.shaikkhaja and Dr.S.S.Panda made a study of cases of OPC poisoning including age, sex, religion, marital status, occupation, seasonal variation, diurnal variation, serum cholinesterase levels and associated diseases. serum cholinesterase levels done by kinetic calorimetric method[22,23].

It was explained that out of thirty five cases of OPC poisoning twenty seven victims were between 15-30years,next 31-60years, and least in the age group 1-5years. 25 cases were male victims including a male child and 10 were female victims[22,23].

Among the males 4 of them were unmarried and the rest were married. Among the females 5 of them married and 5 were unmarried. 28victims were from the rural areas and 7 from the urban area.17 victims were farmers by occupation , 7were unemployed, 5 were house wives, 2 were student victims. 32 victims consumed during day time 6AM to 6PM.He also showed that the

levels of serum cholinesterase levels OPC poisoning death cases were very low upto 878 U/L.

Victims consumed 250-500ml based on the history given by the victims and their relatives. Incidence of deaths were 22.85% 3-6 hrs period of survival, and the serum cholinesterase levels were 70u/l to 878u/l. survived male victims had 814-9056 u/land female victims had 1978u/l to 9240u/l. victims came for medical treatment mostly 3hours to 6hours after consuming. Mode of poisoning were suicidal followed by accidental and no homicide were reported[23].

The peradeniyaorganophosphorus poisoning scoring system scale was done by N.senanayake,H.Jdesilva and karallieddein1993. The score obtained with clinical manifestations of pupil size, respiratory rate, heart rate, fasciculation, level of consciousness, and seizures. Pupil size:>2mm-0,<2mm-1,pinpoint-2 Respiratory rate:<20/min-0,>20/min-1,>20/min with cyanosis-2 Heart rate:>60/min-0,41-60/min-1,<40/min-2Fasciculation: none-0,Present,generalized or continuous-1,Both generalized and continuous-2 Level of consciousness: Conscious and rationale-0, impaired response to verbal commands -1 [23].

No response to verbal commands-2, seizures: absent-0,present-1. Mild poisoning score was between 0-3, moderate poisoning score was between 4-7, severe poisoning score was between 8-11. Correlation was made between pop scale and serum cholinesterase level[23].

A P value of less than 0.05 indicated the significance. The serum cholinesterase levels in mild poisoning cases 35(70%)were 2647u/l, moderate poisoning cases 13(26%)were 200.2u/l severe poisoning cases 2(4%)were 124.5u/l. The study observed that there was a significant correlation between derangement of serum cholinesterase level and severity of poisoning. The deranged serum cholinesterase level was associated with increased requirements of atropine and prolonged duration of hospital stay. The mortality rate was 14%.The other factors like pneumonia, septicemia, cardiac arrhythmias, broncho constriction depression of the respiratory centre resulted in deaths. The study concluded that serum cholinesterase levels were useful in assessing the severity, amount of atropine needed and duration of stay in hospital for the management[23].

Fratello et al reported a case of acute OPC poisoning by parenteral route[24].A 33years old male attempted suicide by injection of phoxim (10ml) in the distal region of his left arm. The patient was conscious B.P-120/75mmhg, pulse 85/min, pupils 2mm with a positive light reflex. Respiratory rate was 25/min. Examinations ofnervous system was normal[24].

Examination of abdomen was normal. Serum cholinesterase level was 200Iu/L(normal range 5900-12,220) Patient was treated with atropine, pralidoxime, I.V crystalloids for 15 days. The signs and symptoms did not did not manifest immediately due to the low level of OPC distributed to the circulatory system. Here the measurements of serum cholinesterase level was useful for confirming the exposure[24].

Patient did not recover in spite of continuous treatment and his serum cholinesterase level was low. He accidentally scratched his wound and there was bursting of bloody liquid which had an odour of insecticide [24].

Surgical debridement was done and patient improved and his serum cholinesterase level increased to 3823IU/L [24].

The mortality associated with serial cholinesterase activity was done by Fisher's exact test and was shown to be statistically significant.(0.038) out of the 8 deceased among the 55 study group3of them had elevated Sche level and 5of them did not have elevated Sche levels. Among the survival group of 47 individuals, 36 of them had elevated Sche levels and 11 of them did not have elevated Sche level. It was estimated that the mortality rate was 11-23% following organophosphorusingestion.

It was reported by Nouria et al that plasma cholinesterase level on the day of admission had no correlation with the degree of poisoning. Sche level for thirty patients with organophosphorus poisoning had no prognostic value. In contrast it was reported by others that plasma cholinesterse is useful in early detection of organophosphorus toxicity. Scheis variable from person to person.

Hereditary deficiency of the enzyme, malnutrition, iron deficiency anemia, liver function, drugs like cocaine, morphine, codeine andsuccinyl choline are the variable factors making this enzyme less reliable if the baseline levels are not known.

It was reported by Eddleston et al that various types organophosphorus compounds have different chemical characteristics and their poisoning outcomes also differs.

The mortality and pseudocholinesterase inhibition by chlorpyrifos is not applicable to dimethoate.The mortality rate in organophosphorus poisoning is associated with the absence of increase in serum cholinesterase levels.

A study of clinical profile , cholinesterase level of 52 patients with OPC , carbamate, OPC, carbamate mixed and misc. poisoning was done by Goswamy, chaudri, manashun .It was found that plasma and RBC cholinesterase level were lowest in the mixed group and highest in the carbamate group.

A study was conducted SCB medical college by doctors in the department of medicine to estimate the levels of serum cholinesterase,serum LDH, serumCPK, in cases of OPC poisoning .serum cholinesterase and CPK levels strongly correlated with the severity.

Serum cholinesterase serves as diagnostic marker of OP poisoning and not as prognostic marker.Serum CPK has a strong positive correlation with severity and can be used as a predicted of outcome in OP poisoning. Based on POP score mild poisoning cases had a serum cholinesterase level of 2006.75+/-1554.7 (normal range 4620-11500 U/L in males,3930-10800U/L in females),moderate poisoning had a serum cholinesterase level 1389U/L+/-1554.0U/L, and severe poisoning had 838.70+/-699U/L

Serum CPK in mild poisoning cases were 449.69+/-325U/L(normal range 55-170U/L in males, 30-135U/L in females),moderate poisoning 768.2+/-485.4U/L, severe poisoning1324.74+/-1416.6.

Serum LDH in mildpoisoning cases were 329.0+/-125.47(normal 313-618U/L),moderate poisoning 480.31+/-310.05U/L, and in severe poisoning 497.88+/-321.87U/L. There was a negative correlation in severity of poisoningand serum cholinesterase levels. The serum CPK showed a positive correlationwith the severity of poisoning. Both were statistically significant (p=0.001).The correlation with the serum LDH levels were also positive and wasnot statistically significant.

A study was conducted to investigate and compare oxidative stress, and immune modulatory effects of pesticide exposure among agricultural workers .95 adult males were selected , classified into control group 30 unexposed,55 exposed to organophosphorus and carbamates, and 10 exposed to fungicides. The oxidative stress was evaluated by assessment of SH protein, glutathione-S-transferees, glutathione reductase,total antioxidant capacityandmalondialdehyde. The acetyl cholinesterase was measured as a biomarker of toxicity[25].

IgG, IgM were used as immunological biomarkers, and TNFalpha as a biomarker of cellular immune function. The results statistically revealed significant reduction in the activity of the acetyl cholinesterase , antioxidant defense enzymes, total antioxidant capacity, IgG, and IgM while Malondialdehyde and TNF showed elevations in the insecticide exposed groups.

Among the fungicide exposed groups there was a non-significant reduction in the activity of acetyl cholinesterase, antioxidant defense enzymes, IgM, IgG and TNF alpha levels while there was significant elevation of malondialdehyde level and significant reduction of total antioxidant capacity level[25].

The first reaction of OP is interaction with cholinesterases in the blood stream ("first come first served") (Benschop and de Jong2001) and then in the target tissues the central and peripheral nervous system.(Bajgar,1985,1991,Bardin et al 1994, Green 1958, Marrs et al .1996). However other changes were accompanied with the development of toxicity such as changes in other enzymes, immune changes, and anaphylactoid reaction. Fromthis basic enzyme inhibition mechanism, the first step for prophylaxis is focused on protecting cholinesterase against inhibition or decreasing the concentrations of OP Compounds [26].

Detoxification is by administration of enzymes to break OP-Che complex.Administration of enzymeto bind OP decreases the OP level in the organism. Enzymes to hydrolyze OP are under investigation(li et al.,1995) Buche and Ache were reported to be effective against OP intoxication.(Clark et al., 2002; Doctor et al., 1991,1997,2002, Mars et al.,1996, 1993, 1998, moore, 1996, Saxena et al1997) These enzymes act before before the toxic action of OPs[26].

Prognostic value of serum cholinesterase level in organophosphorus poisoning: 30patients of acute organophosphorus poisoning in a medical ICU were studied prospectively. Severity of the intoxication was assessed by total dose of atropine needed to relieve poisoning manifestations. It was concluded that mean serum cholinesterase level was not significantly different in mild and severe poisoning[27].

It was reported that identifying high risk patients by this measurement was not reliable.Three patients who died after 2 days had variable serum cholinesterase activity 1800U/L,300U/L,100U/L. The cause of death was ventricular fibrillation in one patient and a-v block in two patients[27].

A cross sectional study was done byDevanurR.M.M.Prasad, and his associates at Dept.of Forensic Medicine, Jawaharlal medical college,Karnataka, among 76 patients of OP poisoning to detect the relevance of Plasma cholinesterase to clinical findings in acute organophosphorus poisoning[27].

In their study they reported that that the deceased patients had the lowest plasma cholinesterase levels of 1270U/L. Chen et al showed that low plasma cholinesterase level with non raising trend for 2days of OP poisoning was associated with higher mortality.Eddleston et al revealed that plasma cholinesterase activity can be used to predict death based on the formula of the OP compound ingested. Karr in 2006 and Tsao et al in 1990 showed that fatal out comes following OPcompounds poisoning were associated with lower plasma cholinesterase levels[27.]

Cases ofpaediatricorganophosphorus poisoning manifest with hypotonia, lethargy, coma, and seizures. This may be contributed to diagnose as respiratory infections, viral syndromes, or drug related encephalopathy.Diagnosis is based on history of exposure, and consistent symptoms. In suspect cases, blood samples to be drawn for plasma pseudo cholinesterase levels and RBC cholinesterase levels. Lowering of the enzyme levels occurs within minutes of significant absorption of OP compounds.Depression of the plasma enzyme for several days to weeks.RBC value may take several days to reach its minimum remains same as long as 1-3 months, until new enzyme replaces that inactivated by organophosphorus[27].

Individual base line cholinesterase levels are variableto person to person. To confirm the suspected OP poisoning,20% of the depression of the plasma cholinesterase level is necessary.The body hydrolyses organophosphates producing alkyl phosphates and phenols that may be detected in the urine 48 hours after absorption.Urinary alkyl phosphates and phenol detection may be possible at lower dosages of OP absorption than that required to depress cholinesterase activities and signs and symptoms of toxicity[27].

VenkateshwarluN, Professsor and HOD, Dept. of General medicine, SVS Medical collegeAP,India and his associates made a studyof 200 cases of OP poisoning. The severity was assessed clinically and by serial estimations of serial serum cholinesterase levels.Serum cholinesterase inhibition was a an indicator of severity and diagnosis of the disease but improvement in the levels may not be to the extent of clinical improvement . Nigghnknak formed

an opinion that blood cholinesterase levels as human biomarkers of Op pesticide exposure. The estimated serum cholinesterase levels in mild poisoning were2240 U/L, Moderate 1640U/L,severe 1008U/L. Total of 22 patients expired within 2days of admission[28].

Their follow up study revealed that clinical improvement was 94%, where as biochemical improvement was 58%[28].

Several factors have been responsible for the severity of the OP poisoning like dose of the poison, formula of the compound ,time of the treatment. They reported that serum cholinesterase level was a sensitive indicator in assessment and prognosis[28].

Even in the recovered cases there was no spontaneous increase in serum cholinesterase levels. It is known that after the delay of 36 hours, the enzyme inhibition in irreversible. Inhibition of the enzyme even after recovery was a feature in some cases. Absence of recovery of RBC and plasma cholinesterase levels in expired patients could not be confirmed as the patients expired quite early[28].

Atropine and pralidoxime aided by serum cholinesterase levels afforded excellent prognosis. The time interval between the ingestion of poison and treatment is directly proportional to the severity. The patients who recovered showed increase in serum cholinesterase levels[28].

Dr.Khaimudabbir Ahmed, Assistant Professor, Dept of Forensic Medicine and Toxicology made a study to correlate the clinical score described by Peradanyaorganophosphorus poisoning scale, serum cholinesterase level, to mortality among organophosphorus poisoned patients.64 patients were included. The severity of the poisoning by POP scale correlated directly serum cholinesterase level. Deaths in serum cholinesterase level <10% were 10 , 10-20% - 1, 20-50% - 1[28].

A correlation was also observed between the deranged serum cholinesterase level and mortality and morbidity of the patients with prolonged duration of the hospital stay. 85% of the patients who died were in moderate and severe poisoning group[28].

Deaths in cases of mild poisoning were contributed to co morbid conditions like pneumonia, septicaemia, and cardiac arrhythmias which also have a role in mortality after OP poisoning[28].

Deaths among OP poisoning are due to depression of CNS respiratory centre, neuromuscular weakness, excessive respiratory secretions, broncho constriction, and cardiovascular collapse.10% of the patients who had taken 120 ml or more of poison had a lower cholinesterase levels with high mortality rate. Death was also associated with more time interval between consumption and hospitalization. Lower serumcholinesterase level was associated with death outcome[28].

Sachincolak ,Dept. of emergency medicine, Haydarpasanumune training and research hospital made a studyto evaluate the association of plasma glucose

and serum cholinesterase level as predictors OP induced intermediate syndrome. 71 patients of OP poisoning were evaluated for a period of 4years.The serum cholinesterase level in patients without intermediate syndrome were 2651+/-1266.68.U/L where as the levels in patients intermediate syndrome were465.11+/-302U/L. Intermediate syndrome(IMS) is a clinical syndrome characterized by respiratory muscle paralysis, proximal muscle weakness, motor cranial nerve involvement which is associated with mortality[29].

Rational use of cholinesterase activity testing in pesticide poisoning was reported by James.E.lessenger M.D and BenjaminEReesePhd.The use of cholinesterase testing in pesticide poisoning becomes a burden to the family physician in evaluating a suspected poisoning case. A23% variance in acetyl cholinesterase level exists even among normal individuals[30].

It is therefore essential to establish the baseline levels to avoid the risk of diagnosing as pesticide exposure. The employers who are monitored with the baseline levels, the diagnosis can be made by comparing the baseline levels with the post exposure levels. If the baseline levels are not known, the offending chemical is in question , the treatment is based on signs and symptoms. Direct measurement of pesticide levels in blood or urine is expensive and cumbersome. Each pesticide mayneed a separate assay and serum level of the chemical may not be related to the degree of enzyme inactivation[30].

Even proved exposures the blood pesticide level may be too low for detection. Therefore cholinesterase activity testing has an advantage of measure

of physiologic response.Medications affecting the neuro muscular junctions can alter the cholinesterase levels [30].

Virennaik, and Ashvinmevada made a study to evaluate the clinical profile and plasma cholinesterase activity in OP poisoning at civil Hospital Ahmedabad among 50 patients [31].

All the patients at the time of admission had a lower plasma cholinesterase level of 2.83+/- 0.9 Ku/l. against the lab standard of 5.1ku/l-12.1ku/l (adult male and post-menopausal women) and 4.1 -11.9(adult pre-menopausal women) Patients who consumed more than 200ml of OP poison and the plasma cholinesterase level less than 2.0ku/l were associated with death. No deaths were reported in patients who consumed less than 200ml of OP poison and serum cholinesterase level above 2.0ku/l [31].

The values increased from day 3 and continued in surviving patients. Within one to two days of OP binding to acetylcholinesterasesome phosphorylatedacetylcholinesterase can be dephosphorylated and become reactivated by oxime antidote. As time progresses the enzyme phosphoryl bond is strengthened by loss of one alkyl group and reactivation by PAM is not possible [31].

The total deaths were 11 and all of their plasma cholinesterase levels were less than 2ku/l. There are also ethnic differences in the extent, type, and the amount of poison consumed and the utility of diagnostic measures [31].

Liu et al did a retrospective study in OP poisoned individuals to determine whether GCS(Glasgow coma scale), serum cholinesterase levels,and leucocyte

levels had prognostic value in acute organophosphorus poisoning. The study was done in patients at ICU selcuk university ,emergency department, Konya in2006-2009.The mean GCS values of patients who died were significantly lower than who survived (4vs11). The mean serum cholinesterase levels in those who died were lower though not statistically significant.(1768vs3841u/l) The highest leucocyte level was 18.6k/ul among the died and the mean leucocyte level among the survivors 13.4k/ul. Although serum cholinesterase level may be used in the quick diagnosis the efficiency in predicting the out comes in OP poisoning is not established [31].

The outcomes are reveal that the serum cholinesterase levels and leucocytes values are of diagnostic value .They do not have prognostic value. All patientswho died had higher leucocyte levels [31].

In a study conducted by cherian et al on 21 patients with OP poisoning, no significant difference in levels of serum cholinesterase was noticed between the group treated with Pralidoxime and the group who received placebo. But it was observed that mean serum cholinesterase levels were lower in patients who died [31].

Other lab findings include haemoconcentration, metabolic and respiratory acidosis, hyperglycemia, hypokalemia,hypomagnesemia, elevated amylase, elevated troponin levels, and elevated liver function tests[31].

A study was done at National poison control centre, Karachi on patients with organophosphorus poisoning from Jan1999 to Dec2002[31].

All of them were monitored with serum cholinesterase levels. The results showed that 44.97% had low level of serum cholinesterase, 33.21 in the threshold range, 13.10 % had above normal range. It was stated that it is of less significance unless the baseline level is not known. It was reported death was associated with decrease in serum cholinesterase activity[31].

Vivek A chiddarwar and his associates did a study on clinical profile of house hold and agricultural insecticide poisoning patients with reference to serum cholinesterase levels. Total no. of cases in 74% compared with other insecticides.vomiting and salivation were the predominant symptom followed bysweating.Altered sensorium,pain abdomen, incontinence, seizures were other manifestations.Among the patients who were hospitalized in less than or at 2hours recovery were 16 and deaths were 4. Among the patients who were brought at more than 2hours recovery were 17 and deaths were 13. Which shows the longer time available for absorption of OP compounds leading to fall in serum cholinesterase levels[99].

On comparing the serum cholinesterase levels in Patients who recovered and died ,those who recovered had a mean serum cholinesterase levelof 2183+/-1607u/l Those who died had the serum cholinesterase levels 2237+/-1218, p value >0.05.They concluded that there is no association between serum cholinesterase levels. Kishore et al reported that recovered patients had a mean serum cholinesterase levels of 4092+/-4704.2.Dead patients had a mean serum cholinesterase levels of 3900+/_- 4617u/l. p value >0.65.Mehta et al

reported that two patients with severe suppression of cholinesterase level less than ten percentsurvive.Dua et al studied 43 patients of Op poisoning and reported that neither mortalities,nor the clinical severity correlate with serum cholinesterase levels[99] .

A study was done in the department of anaesthesiology and critical care based on the effectiveness of the pralidoxime in the treatment of Organophosphorus poisoning. After the institutional review board approval, 100 patients with clinical evidence of OPP or report by relatives of poison ingestion were included.Patients with Bardin grade2 who had hypotension and deteriorating consciousnessand Bardin grade 3 abnormal x-ray chest and stupor were shifted to ICU for pralidoxime therapy that is to be done at ICU.Age less than 14 and more than60 , known pregnancy, pralidoxime therapy before ICU ,carbamate poisoning, chronic illness, treatment started after 12 hours of consumption of poison were excluded. All patients brought to the hospital were managed by standard OPP protocol of decontamination,air way resuscitation,breathing and circulation.Atropine was given and titrated to the target end points[41].

Age,sex, type of the compound ingested,severity of the poisoning, cholinesterase levels at the time of admission, 1,3,5 days,day of discharge or at death were recorded. All patients were given atropine two to three mg on arrival. The dose was doubled every five minutes till atropinisation.

Signs of atropinisation is indicated by heart rate>100/min,dry mouth,clear lungs on auscultation, normalization of bowel sounds, and mid dilated pupils.Pralidoxime was given to the study subjects in group (AP) according to W.H.O guidelines.30 mg/kg/h continuous infusion for 7days . Maximum period of acetyl cholinesterase inhibition is presumed to be seven days in most of the patients.clinical improvement such as resolution of muscle fasciculations, weaknessand absence of atropine requirement were also considered indicating the presence of sufficient cholinesterase levels at the synapses.Placebo group received IV saline .Atropine was withdrawn slowly after aperiod of three to five days[41] .

Results were reported as : The blood pressure and heart rate did not differ in both groups. In group A, among the survivors,cholinestersase on admission were 1476.9+/-431.2 and in nonsurvivors 916+/-311.7($p>0.05$).The cholinesterase levels (mean+/-SD) at discharge among the survivors were 4516+/-976.2 and in nonsurvivors were 3170+/-1503 with ($P<0.05$)[41] .

In AP group among the survivors,cholinesterase levels (mean+/-SD) on admission were 1316+-481.1 and among the non-survivors the levels were 915.1+/-544.8.($p<0.05$). The levels at discharge in survivors were 4456.4+/-563.9 and in non-survivors were 1463.8+/-576.4[41] .

The incidence of intermediate syndrome was not statistically significant between the two groups.(p>0.05).There was no statistical difference in total dose of atropine required for both groups. 60 patients were in need of Ventilatory support. 31 of 50 patients (62%) required ventilator support in group AP and 29of 60 (58%) in group A [41] .

The development of complications were not different inboth groups.(p>0.05)Pneumonia was the frequent complication in 34% of the patients in group AP and26% in groupA. The other complications in AP group were 22% of cardiac dysrhythmias,8%, of non-cardiogenic pulmonary oedema, and 6% urinary tract infections.The other complications in A group were cardiac dysrhythmias 19%,non cardiogenic pulmonary oedema4%, and urinary tract infections 12%.

Duration of ventilation (mean+/-SD) was 3.5+/- 4.6 days in AP group and 3.6+/-4.4 days in A group.There was no statistical significance P 0.08 in one way analysis of variance.Mean duration of ICU stay in days were also not statistically significant.P.0.05, with7.1+/-5.4 in group AP and 6.8+/- 4.7days in group A [41] .

Ingestion to treatment interval was also not statistically significant with(p>0.05) 5.8+/-3.2 hours in AP group and 5.5+/-2.8hours in Agroup.Case fatality rate was slightly higher in group A 28%and in group AP26% with over all mortality rate of 27%.There was no statistical significance in one way analysis of variance [41] .

Mortality was 42% in group AP and 48.2% in group A seen only in ventilated patients.leser duration was the period of ventilator support in patients who survived than who died ($p<0.05$).

It was concluded that Pseudo-cholinesterase levels correlated with severity of poisoning but lacked prognostic significance.There was a progressive increase in Pseudo-cholinesterase levels in survivors of AP groupbut in non-survivors the change was minimal.Eddle ston et al studied thatreactivation of red cell acetyl cholinesterase may not be essential for survival.They reported that their study revealed reactivation of plasma cholinesterase levels had positive correlation with three fold rise in the base line against the minimal in the patients of non-survivors of AP group [41] .

The two groups did not differ much in their mg of atropine requirements. Haemodynamic requirements did not differ much in the two groups.Tracheostomy was necessary in two patients of group A Hypernatremia was present in36% of the patients on admission.The out comes were worse with increased serum sodium on admission.Hypernatremia was associated with five fold increase in mortality. The complications were Pneumonia,dysrhythmias. Hpoxia, acidosis and electrolyte disturbances were the precipitating factors.

A prospective study was done by Abbasagabiklooei, department of Forensic medicine and Toxicology and his associates among 24 patients . total number of males were 17 and females were 7. There was no difference in the initial symptom

of admission,amount of poison ingested,duration of stay in the hospital,respiratory rate ,pulse rate,temperatureand cholinesterase levels in the survivor and non survivor group.There are limited studies about cardiac injury and intoxication.They are associated with cardiac arrest, cardiac arrhythmias.Their study was to determine the myocardial damage in organophosphorus poisoning. Peripheral arterial blood gases,creatine kinase, creatine kinase myocardial band.TroponinTwere performed inallcases. Non-survivors had highsystolic blood pressure, longer duration of mechanical ventilationand lower gcs scale[51] .

The serum markers of cardiac injury ,initial and later parameters in survival group and non-survivor group wereas follows: the amount ingested-303.2+/-152.9, vs 412.2+/-178.3 not significant.Glasgow ComaScale on admission 10.6+/-2.74 vs14.1+/-0.76(P0.001)

Serumcholinesterase levels 303+/-50.9 vs 412+/-45.9(P0.17)

Creatine kinase:246.9+/-114.9 vs 133.20+/-84.1(P0.025)

tropononinI8.57+/-4.9vs3.98+/-11.65(P0.001)

Creatine kinase-Myocardial band148.64+/-103.9vs17.7+/-29.1(P0.001)

Duration of stay in the intensive care unit(days)4.5+/-2.6vs2.27+/-1.5(P0.025)

Partial pressure of carbon di oxide-59.6+/-7.1vs50.8+/-4.475(P0.003)

Duration of stay in Hospital(days)-4.6+/-2.4vs4.27+/-1.8(P-notsig)

Partial pressure of oxygen72.7+/-8.8vs89.4+/-4.5(P0.001)

Mean duration of mechanical ventilation in hours-3.7+/-1.56vs1.07+/-0.26(P0.001)

Respiratory rate /min30.5+/-8.8vs 31.5+/-4.9(NS)

Pulse rate /min:45.5+/-7.6vs51.73+/-8.3

Temperature36.9+/-0.40vs36.8+/-0.17

PH7.17+/-0.10vs7.28+/-0.04(P0.003)

Hco3(mmol/l)17.1+/-2.26vs20.6+/-1.8(0.001)

ECG did not show any myocardial necrosis in pesticide poisoned patients..Yavuz and his associates reported that increased leves of tropninT level found in patients organophosphorus poisoning.They also reported that there was no significant difference the serumcholinesterase levels in the survivors and deceased.

It was shown that organophosphorus poisoning caused rise in lipid per oxidation products.Ther was rise in the serum markers of heart injury in organophosphorus Poisoning[51] .

As organophosphorus finds in place in the agricultural and domestic areas in the world self .poisoning is not uncommon.in the world. It also finds its place in usage in warfare and terrorist attacks.The mechanism of action is by acetylcholine inhibition in the body.The excess of acetyl choline cause constant triggering and impaired function of the somatic, autonomic and central nervous systems.The clinical manifestations are cholinergic crisis.The para sympathetic stimulation predominates and over stimulation of the nicotinic receptors are due to excess of acetyl choline which leads to sympathetic activity.The 2nd manifestation is the intermediate syndrome which is manifested as cranial nerve palsies,weakness of the neckand paralysis of the respiratory muscles[55] .

The toxicity of the OP is not limited to acetyl cholinesterase binding and are highly toxic. They bind to other enzymes and cause delayed neurological

symptoms.carbamates are also structurally related to OPs and cholinesterase inhibitors.They also produce clinically similar symptoms as that of OPs but are milder. With shorter duration of action[55] .

Some case reports are, Acommunity hospital referred a 37year old farmer on 9thday of unknown quantity of self ingestion of parathion .Intubation was done to him.Treated with 250mg of obidoxime IV as abolus,infusion was given 50mg/hour.Atropine 4mg/hour was given intravenously. After extubation on the second day, he again developed respiratory difficulty and was re-intubated..Antibiotics were given. On the eighth day of ventilation his pulmonary was worse.He was referred as the case of ongoing OP toxicity. Sedatives,dopamine 1.5micrograms per kg min , atropine4mg/hour,were administered. His blood pressure was normalwith heart rate of 90/min.His temperature was 38.5 deg centigrade. Obidoxime was given50mg/hour, Potssium chloride, sulfentanil, ipatropium, salbutamol, enoxaparin, cefuroxime, tobramycin,and trimethoprim-sulphamethoxazole were given.Bilaeral infilterates were the findings on the x-Ray chest. His laboratory reports were Hyperkalemia(6.7mmol/l(,normal range 3.6-4.8mmol/l), renal failure with creatinine values of222micrmols/l, (normal range62-110micro mol/l),urea 15.1mmol/l(normal range 3.2-6.8 mmol/l) and cholineaterse level was 140u/l(range3700-11000u/l)Arterial blood gas analysis had pH 7.30(range7.33-7.45),Pco225.7KPa(range9.2-13.9)bicarbonate 2.2mmol/l(range 21-25),and oxygensaturation was 99%(normal range96-100)Other laboratory parameters were normal.His electrocardiogram showed no conduction abnormalities.

Hyperkalemia and renal failure could have been due to trimethoprim.Then potassium supplementation and nephrotoxic drugs were stopped.Atropine infusion was lowered..sufentanil was stopped and midazolam and morphine were substituted.sputum culture did not reveal any pathogens. Amoxicillin with clavulanic acid were administered.Obidoxime was stopped.Renal functions improved,ventilator support was tapered and was extubated on 12th day ofadmission. On13thday., atropine was stopped.He was discharged on14thday. He was followed up in the Medical ward with Psychiatric follow -up. The complications were ventilator associated pneumonia,and renal failure. Hyper thermia was due to over atropinisation.But other signs such as tachycardia and ileus were absent[67] .

Patient B:A man of aged61years was admitted in the ICU with a history of alcohol abuse, depression,and suicidal attempts.The history was ingestion of unknown quantity of parathion following which he lost his consciousness. Cardiac asystole was noted by the paramedics and cardiopulmonary resuscitation was given.After ten minutes of resuscitation circulation was achieved. Aspiration of the gastric contents was a possibility and he was transferred. He was comatosed and was ventilatedHis pupils were pinpoint with no response to light,, Heart rate 105 beats /min, fasciculations were present. There were no abnormalities in physical examination. Laboratory values were leucocytosis of 18.6x10 ^{9} /l(Range: 4 to 10x 10^{3}/Cub. Mm) and an elevated lactate of12.5mmol/l(range 0.5 -2.2mmol/l) liver function tests were abnormal with lactate dehydrogenase610u/l(range114-235u/l),aspartate aminotransferase87u/l(range upto 40u/l),alanine aminotransferase 51u/l(range51u/l)and gamma glutamyltransferase 64u/l(range upto65u/l) and blood

gas analysis showed pH of7.1,pco25.5kpa,po224.1kpa , bicarbonate was 12mmol/l and oxygen saturation was 98%. Showing metabolic acidosis. His blood cholinesterase level was not detectable. There was no abnormalities in his x-ray chest. No abnormalities in his electrocardiogram. Atropine was administered 1mg/hour IV after bolus dose of 2mg and 0.5 mg. Activated charcoal and 10mg of diazepam were also given.Obidoxime boluses of 250 mg was given. Rocuronium was given to treat fasciculations. He then developed sepsis and coma. Electro encephalography was done on 17th day showed little activity. No cortical activity was detected on somato sensory evoked potentials testing. The patient died on the 18th day. His death was contributed to prolonged anoxia during the initial phase[67] .

Patient C:A patient of 63years old ,farmer by occupation, with a history of depression,admitted to the hospital after consuming 200ml of parathion several hours before. His family members brought him for medical services on seeing him unconscious. The paramedics noticed dilated pupils, bradycardia, (32beats/min) bronchorrhoea, in a comatosed person. Intubation was done and started on atropine and obidoxime. His blood pressure was 155/85mm.hg.and pulse rate was110/min.Blood gas analysis revealed pH7.11,pco28.5kpa, po257.2kpa, bicarbonate 20mmol/l and o2 saturation 99%.There were no physical abnormalities on physical examination. Serum parathion level was 800microgram/l.(toxic above 10microgram/l),Serum cholinesterase level was not detectable indicating severe intoxication .Activated charcoal was given,.sedation and propofol were administered. Obidoxime 40mg/hr was given IV and stopped.Atropine was given 3mg initially and titrated. He then developed aspiration pneumonia, and bactremia

with klebsiella species. On 8th day he was extubated.,Atropine stopped on 9th day,restarted due to reappearance of bronchorrhoea. Atropine was stopped on 11th day. He was discharged to medical ward on 12thday. His cholinesterase level raised to2000u/l.He was discharged to psychiatric ward on 20th[67] .

Intoxicated patients should be resuscitated with maintanenceof circulationand ventilation. The diagnosis of Op intoxication was obtained from historyand confirmed by the clinical picture, and reduced serum cholinesterase levels. All signs and symptoms were not present in every patients. If the diagnosis is indoubt,estimation of plasma cholinesterase(also called pseudocholinesteraseor butryl cholinesterase) or erythrocyte cholinesterase is useful.The plasma cholinesterase is an indirect biomarker of acetyl cholinesterase inhibition and there is no direct relation to the inhibition of acetylcholinesterase in the synapses. It is of use to detect exposure to organophosphorus compounds.Acetyl cholinesterase inhibition is in the synapses is assessed by estimating erythrocyte acetyl cholinesterase. There are complex mechanisms between erythrocyte acetylcholinesterase and inhibition of nervous system acetyl cholinesterase. The level of erythrocyte acetylcholinesterase is a marker of severity of OP poisoning. In acute cholinergic syndrome, atropine is the treatment of choice. Onset of action is within minutes and has a half life of 2-5hours.There are protocols following bolus doses at fixed time intervals. Doubling the dose of atropine every few minutes is also being followed which reaches doses up to 100mgs.Atropine competes with acetyl choline at the muscarinic site for acetylcholine receptors[67] .

Some other parameters for drug titration are miosis, perspiration,hypotension,bradycardia,bronchorrhoea,bronchospasm..Secondary complications are due to hypoxia,pneumonia,atropine over dose, which present with confusion, hyperthermia and ileus. As atropine is a selective muscarinic antagonist it has no effect on neuromuscular junctions and muscle weakness.

In one patient (C)the parameter for re-intubation was bronchorrohea. Aregimen of atropine 3mg twice daily was followed to establish sufficient control. Knowing the half-life of atropine is two to five hours it seems illogical to follow twice a day regimen. The dilated pupilin one of the patient was due to sympathetic over activity.But his other clinical picture was that of parasympathetic hyperactivity[67] .

In another patient (A) atropine was discontinued early and had to continue long as some of the clinical signs were contributed to OP and not atropine and complications due to ICU treatment. Atropine may be required for weeks.The type of OP consumed, amount ingested and other patient related pharmacokinetic parameters determine the duration of treatment.There are no clear schedules for atropine maintenance and withdrawal.Reduction of atropine dosage according to the development of clinical signs is one of the option. Glycopyrrolate can be used instead of atropine though it is less effective. Seizures and agitation are managed with benzodiazepines.Oximes usage in the treatment of OP is debated .The serine hydroxyl group of the acetyl cholinesterase enzyme's active site is phosphorylated by the OPs. It is reactivated by hydroxyl ion and by removing the phosphorylated serine residue. However, the enzyme is prone for a process of ageing where one

alkyl of the phosphorylated enzyme is replaced by hydroxyl group making the acetyl cholinesterase negatively charged. The ability of the obidoxime and pralidoxime is the ability to catalyse the regeneration of the acetylcholinesterase from the inactive acetylcholinesterase by removing the phosphoryl group. The window period is restricted for this regeneration.Every OP has its own ageing time. There are reviews which failed to find evidence the benefit of oximes than the harm. There are methodogical weaknesses ,under dosing of oximes in most trials.

A randomized control trialof 200 patients revealed that moderately severe Op poisoned patients showed lowered morbidity and mortality when treated with high dose pralidoxime (1gm/hour for 48 hours after a loading dose of 2gm) on comparison with low dose bolus.The trial dosing regimen was suggested by W.H.O in a dose of 8-10mg/kg /hour of pralidoxime after a loading dose of 2gm[67] .

Gastric lavage and activated charcoal are used though it has no proven benefit. To minimize the extent of toxication , skin decontamination with soap and water is used[67] .

There are symptoms associated with occupational exposure, but an actual secondary intoxication has never been proved.The acetyl cholinesterase levels were never measured in affected health care workers. The organophosphorus compounds which are dissolved in highly volatile foul smelling solvents caused more complaints than the nonvolatile compounds themselves.

Heath effects:Prolonged exposure or repeated contact with organophosphorus may result in the effects similar to symptomsof the acute exposure. Workers repeatedly getting themselves exposed to OP compounds

include impaired memory and concentration, disorientation, depression, irritability, confusion,headache,delayedreaction time,night mares, sleep walking,drowsiness, and insomnia.

A 2010 study showed that organophosphorus exposure is associated with the risk of Alzheimers disease. Another study showed that antenatal mothers who were exposed to OPCs had children with attention disorders. Prenatal exposure to OPCs had a significant impact on birth weight and gestational age was shown by study of environmental health perspectives in 2012.

Pesticides kills the useful insects of the soil, and reduces the natural fertility of the soil, leading to virulent and resistant species of insects. Pesticide limits have not been fixed for water and contamination of rivers, lakes,ground water and drinking water occurs. Raw fruits and vegetables sprayed with pesticide above the safety level leading to the risk of cancer in women and children .

Domestic animals arepoisoned there by contaminating the meat and milk. The pollution monitoring laboratory of the centre for science and environment tested the beverage samples. It reported that organophosphorus pesticide chlorpryrifos was 0.0042 than the safe limit of 0.0001 and pesticide malathion was 0.0137 than the safe limit of 0.0001 causing serious concern.The severity of the pesticide also depends on the absorption, its metabolites, accumulation and persistence in the body. Toxic effects depend on the health status of the individual. Malnourished and dehydrated individuals are

sensitive to pesticides. When two or more pesticides are combined they become more toxic and hazardous [67] .

Use of human butryl cholinesterase as aprophylaxis against Russian Vx: This is under advanced development for pretreatment of organophosphorus poisoning. Their aim of the study was to to find the efficacy of human butryl cholinesterase as pretreatment in Russian VX agent the most toxic OP [68] .

RVX (O-isobutyl –s-(2-diethyl , methyl,methyl phonothioate) is a main V-type nerve agent developed for the chemical warfare in the soviet union. RVX is the most toxic OPs. Comprehensive assessment of exposure to highly toxic OPs need biological monitoring.Their aim was to evaluate the prophylactic efficiency of human butrylcholinesterase by monitoring of blood and brain cholinesterase levels in RVX poisoned rats.They performed the experiment from permission of ethic committee. The selected animals were 180-200gm.maintained at 22+/-2deg.centigrade. Intra muscular administration of V -agents was donein the hind limb after 30 minutes of purified butryl cholinesterase 500u/kg administration.saline injection was done in control animals. The animals were narcotised with carbondioxide blood collected from heart in heparinised tubes. Whole brain was preserved.

As the blood in the brain could contain butryl cholinesterase and not suitable to analyse cholinesterase in the brain tissue. Hence the experimental animals were given 0.9%sodium chloride .The acetyl and butryl cholinesterase were estimated[68] .

The inhibition of acetyl cholinesterase in wholeblood was fast and effective.Pre administration of butrylcholinesterase was not able to protect cholinesterase targeted byRVX. At 6minutes time interval, RVX causes 20% reduction in the brain acetyl cholinesterase activity.Human butryl cholinesterase applied was retained without significant changes in the brain acetyl cholinesterase level after one hour of intoxication. In the first minute of intoxication butryl cholinesterase inhibition occurs.At 6th minute there is 50% of reduction and repaired in10th minute by 10% and 20 min by 20%.Pre treatment with butryl cholinesterase provided 100% survival in1 LD50of RVX. After intoxication, one animal died[68] .

Effective counter measures have been on search for counter measures against Ops poisoning.Bioscavengers are an alternative approach for pharmacologic pre and post exposure treatments.There are no side effects for butryl cholinesterase against 5Ld50 of nerve agent.There was linear correlation between blood cholinesterase and protection against poisoning.There was a decrease in cholinesterase level in the 1st minute of intoxication.500u/kg of butryl cholinesterase was not sufficient in RVX intoxication. Decrease in blood cholinesterase showed severe inhibitory activities of acetylcholine in the peripheral compartment. Further increase can be explained by the absorption of butyl cholinesaterase[68] .

The brain acetylcholine was not altered after administration of butyl cholinesterase and after RVX intoxication.The 1Ld 50 of RVX was able to decrease the cholinesterase 20% in the first 3minutes.The central compartment protected by the cholinesterases is important as damage to the brain is not consistent for survival[68] .

Bioscavenger reduces the nerve agent concentration present in the blood before exposure to very lower levels. RVX does not affect the brain cholinesterases[68].

METHODOLOGY
(Material & Methods)

5. METHODOLOGY (Material & Methods)

After obtaining informed written consent from the relatives of the deceased in the prescribed format, Blood samples will be collected from the cases autopsied at Rajiv Gandhi Government General Hospital. Blood would be withdrawn from the heart with a 21G sterile syringe with needle and immediately transferred to a sterile unheparinised sampling test tube, corked with the stopper and sent for analysis by semi autoanalyser in Toxicology Lab, Rajiv Gandhi Government General Hospital. The samples will be tested blindly that the identity of the individual is unknown.

Inclusion Criteria:

All dead bodies oforganophosphorus compounds poisoning subjected for autopsy

Exclusion Criteria:

All decomposed dead bodies subjected for autopsy

Product / Procedure / Investigation Details:

Following the initial dissection protocol, the sternum is lifted up and dissected out exposing the thoracic cavity. An inverted ‘Y’ shaped incision is made on the pericardium and the heart is exposed. Blood would be withdrawn from the Heart with a 21G sterile syringe with needle and immediately transferred to a sterile unheparinised sampling test tube, corked with the stopper and sent for analysis at Toxicology Lab,Rajiv Gandhi Government General Hospital, by semi auto analyser method. The product used is from Reckon diagnostics P.ltd. Method is Kinetic Propionylthiocholinemethod.

Principle:Cholinesterase hydrolysesPropionylthiocholine to propionic acid and thiocholine.

Thiocholine reacts with 2-nitrobenzoic acid to form yellow coloured 5-thio-2-nitrobenzoicacid.The rate of formation of 5-thio-2-nitrobenzoic acid measured at 405nm, is directly proportional to the cholinesterase activity in the sample.

Reagentcomposition:Reagent-

1:Propionylthiocholine,DithionitrobenzoicacidReagent-

2.Buffer.Reagents to be stored at 2-8deg C. Reagent preparation: Reconstitute the contents of each vial the1.1ml of cholinesterase buffer.mix gently until fully dissolved. Specimen is nonhemolysed serum. Cholinesterase in serum is stable for 17days when stored 2-8deg C and 3months when stored below -20degC.Mix and read the first absorbance at 15secs and second reading at 45 secs.

SEMI AUTO ANALYSER FOR ESTIMATION OF SERUM CHOLINETERASE LEVEL

ANALYSIS

AND

RESULTS

6. ANALYSIS AND RESULTS

Thirty six deceased subjects died from organophosphorus compounds poisoning were analysed of which all the cases were suicidal and no accidental or homicidal were brought .Out of 36 subjects 28(77.77%) were males and 8were females (22.22%) indicating that males predominate the females in ending their lives by deliberate self consumption of OP compounds.

The age wise distribution shows that subjects in age group of less than 20years were 2(5.5%), 21-30years were 7(19.4%) ,31-40years were 9(25%), 41-50years were 6(16.6%), 51-60years were 7(19.4%) and 61-70years were 5(13.8%), accounting for more number of cases were in 31-40 age group, who are exposed to more family stress, financial stress and burden of dependants. Even older people in the age group of 60-70 years were susceptible to end their lives by consumption of poison.

Considering the marital status, more number of married persons of about 29 (80.5%) had consumed OP poison, against the others of single, widow, and widower. This shows that the married persons are exposed to family stress. Three cases of OP poisoning were brought dead and the thirty three were on treatment. Most of them were aware that medical facilities are available but inevitable time lapse was present from the time of poison consumption and the time of arrival to the primary hospital and referral hospital.

Viscera analysis detected OP compounds in 15 (41%)cases of 36 of which 3 (8.3%)cases were brought dead. So a large amount of poison or a more fatal OP compound is being consumed in these cases. The rest of the 21(58.3%) cases fatalities were due to the effects of the OP poison.Viscera analysis detected Op compounds in the blood, brain, liver, kidney,stomach and its contents and intestine and its contents.The control sent preservative did not detect OP compounds.

The serum cholinesterase levels were less than 1000 u/l in -11 subjects(30.5%),1001-2000 u/l -17 subjects(47.2%) ,2000-3000u/l – 4 subjects(11.1%) ,3000-4000u/l-2subjects(5.5%),4000-5000u/l-2 subjects(5.5%),5000-6000-1 subject(2.7%).The mean serum cholinesterase level was 1647u/l.

Number of cases in severe poisoningwere 9 (25%),moderate poisoning were 19(52.7%), and mild poisoning were 8(22.22%).

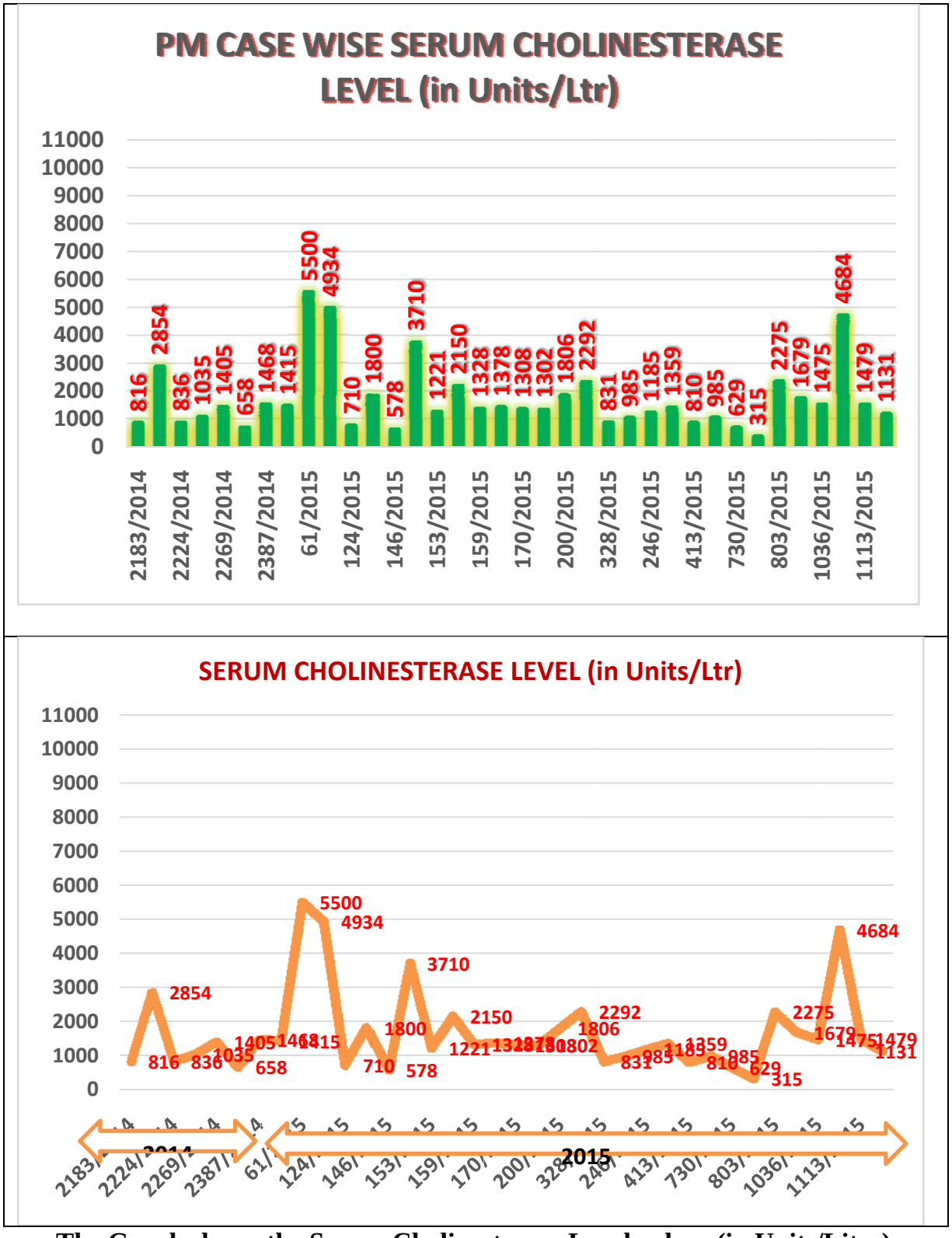
The results are tabulated below;

SL NO	PM NO	Gender	Age (in Years)	Marital Status	Serum Cholinesterase Level (in Units/Ltr)	Lab Standard (in Units/Ltr)	Vicera Analysis Report	Treated/ Brought Dead
1	2183/2014	Male	55	Married	816	6971-11900	DETECTED OPC	BROUGHT DEAD
2	2216/2014	Female	60	Married	2854	6971-11900	NOT DETECTED	TREATED
3	2224/2014	Female	30	Married	836	6971-11900	DETECTED OPC	TREATED
4	2256/2014	Female	28	Married	1035	6971-11900	NOT DETECTED	TREATED
5	2269/2014	Male	38	Married	1405	6971-11900	NOT DETECTED	TREATED
6	2348/2014	Male	48	Married	658	6971-11900	DETECTED OPC	TREATED
7	2387/2014	Male	24	Not known	1468	6971-11900	NOT DETECTED	TREATED
8	2325/2014	Male	24	Married	1415	6971-11900	NOT DETECTED	TREATED
9	61/2015	Male	37	Married	5500	6971-11900	NOT DETECTED	TREATED
10	79/2015	Female	35	Widow	4934	6971-11900	NOT DETECTED	TREATED
11	124/2015	Male	36	Married	710	6971-11900	DETECTED OPC	TREATED
12	145/2015	Male	51	Married	1800	6971-11900	NOT DETECTED	TREATED
13	146/2015	Female	35	Married	578	6971-11900	DETECTED OPC	TREATED
14	152/2015	Male	45	Married	3710	6971-11900	NOT DETECTED	TREATED

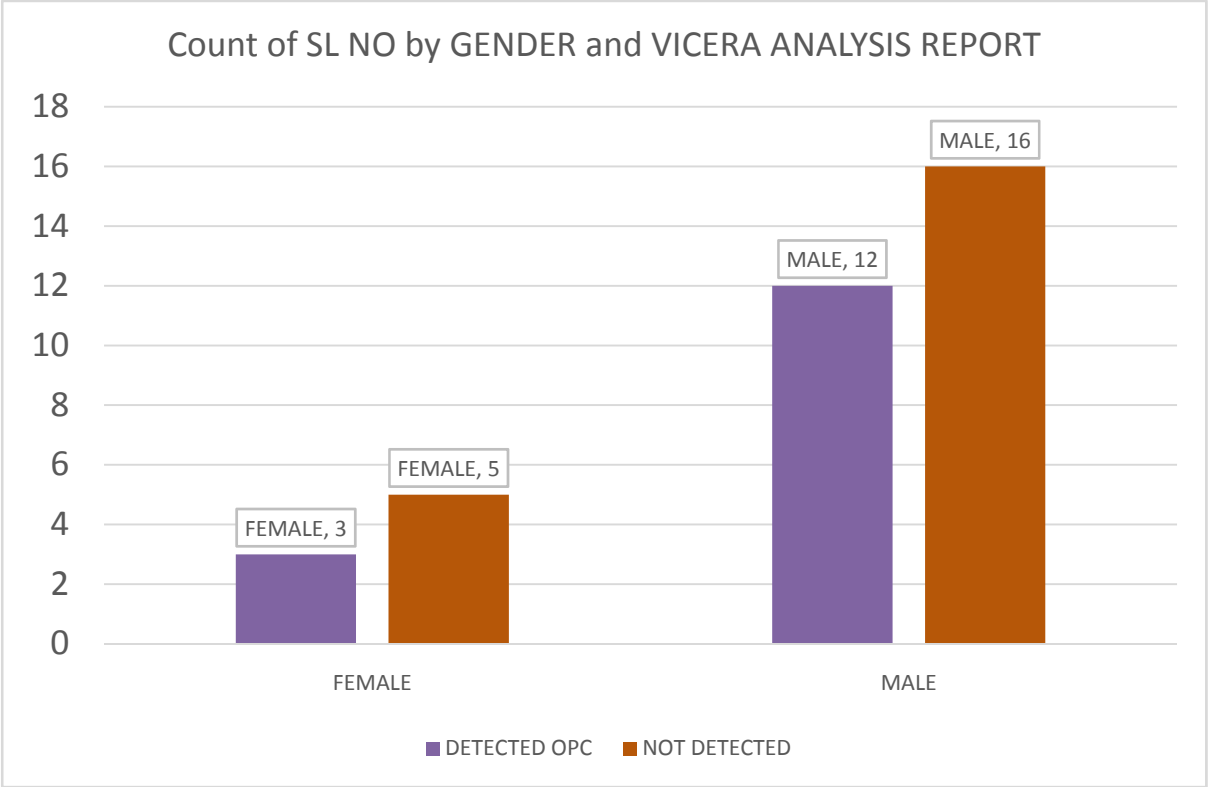
15	153/2015	FEMALE	34	MARRIED	1221	6971-11900	NOT DETECTED	TREATED
16	158/2015	MALE	45	MARRIED	2150	6971-11900	NOT DETECTED	TREATED
17	159/2015	MALE	18	UNMARRIED	1328	6971-11900	DETECTED OPC	TREATED
18	160/2015	MALE	38	MARRIED	1378	6971-11900	NOT DETECTED	TREATED
19	170/2015	MALE	65	MARRIED	1308	6971-11900	NOT DETECTED	TREATED
20	176/2015	MALE	47	MARRIED	1302	6971-11900	NOT DETECTED	TREATED
21	200/2015	MALE	27	MARRIED	1806	6971-11900	NOT DETECTED	TREATED
22	251/2015	FEMALE	23	MARRIED	2292	6971-11900	NOT DETECTED	TREATED
23	328/2015	FEMALE	30	MARRIED	831	6971-11900	DETECTED OPC	TREATED
24	358/2015	MALE	62	NOT KNOWN	985	6971-11900	NOT DETECTED	TREATED
25	246/2015	MALE	70	MARRIED	1185	6971-11900	NOT DETECTED	TREATED
26	248/2015	MALE	45	NOT KNOWN	1359	6971-11900	DETECTED OPC	TREATED
27	413/2015	MALE	47	WIDOWER	810	6971-11900	DETECTED OPC	TREATED
28	447/2015	MALE	55	MARRIED	985	6971-11900	NOT DETECTED	TREATED
29	730/2015	MALE	55	MARRIED	629	6971-11900	DETECTED OPC	BROUGHT DEAD
30	758/2015	MALE	65	MARRIED	315	6971-11900	DETECTED OPC	TREATED
31	803/2015	MALE	52	MARRIED	2275	6971-11900	DETECTED OPC	TREATED

32	963/2015	MALE	18	UNMARRIED	1679	6971-11900	DETECTED OPC	TREATED
33	1036/2015	MALE	35	MARRIED	1475	6971-11900	NOT DETECTED	TREATED
34	1081/2015	MALE	70	MARRIED	4684	6971-11900	NOT DETECTED	TREATED
35	1113/2015	MALE	57	MARRIED	1479	6971-11900	DETECTED OPC	TREATED
36	1169/2015	MALE	35	MARRIED	1131	6971-11900	DETECTED OPC	BROUGHT DEAD

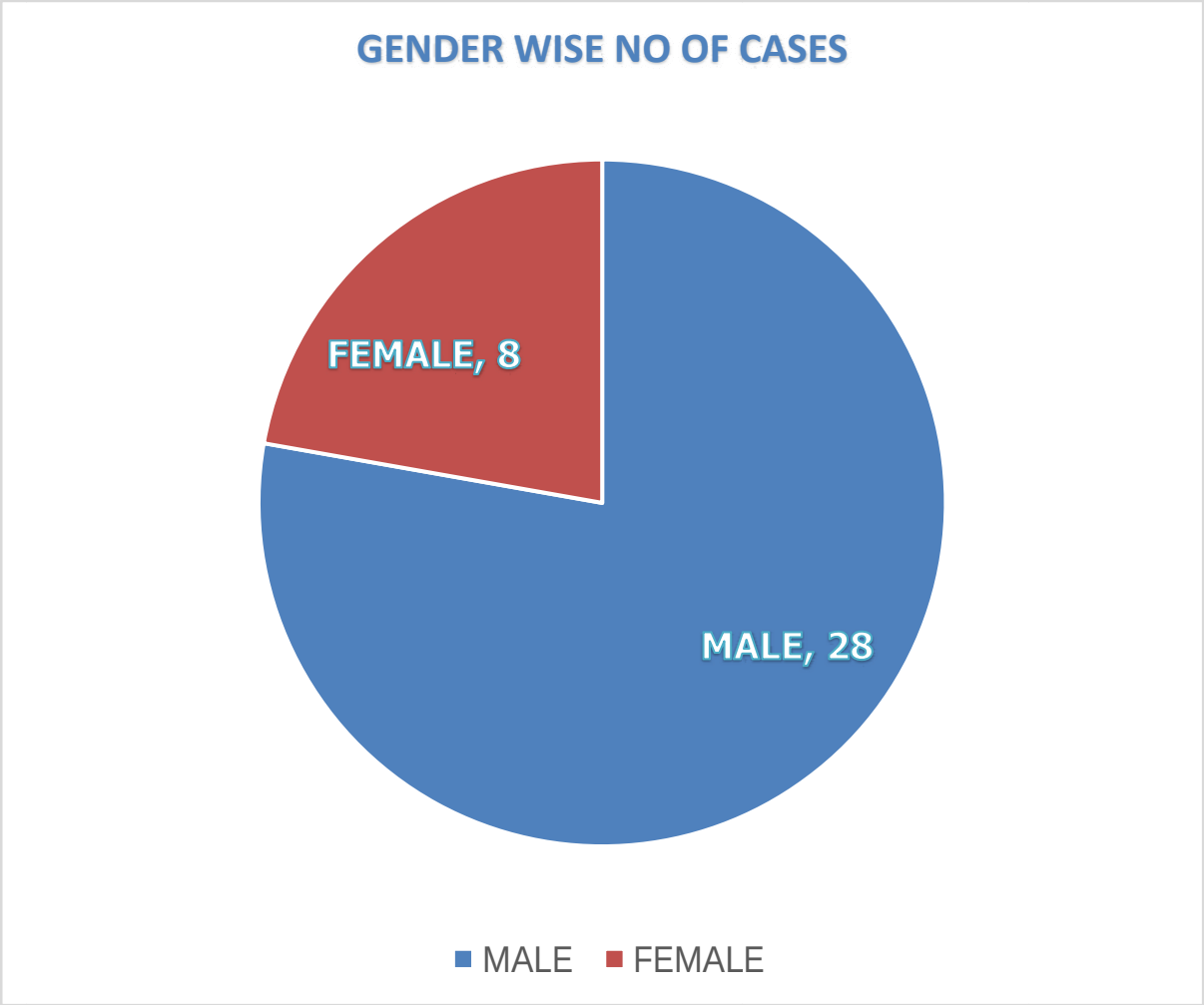
The Graphical representations of analysis from the above table is furnished below;



The Graph shows the Serum Cholinesterase Level values (in Units/Litre) against the Lab Standard values of 6971-11900 U/L



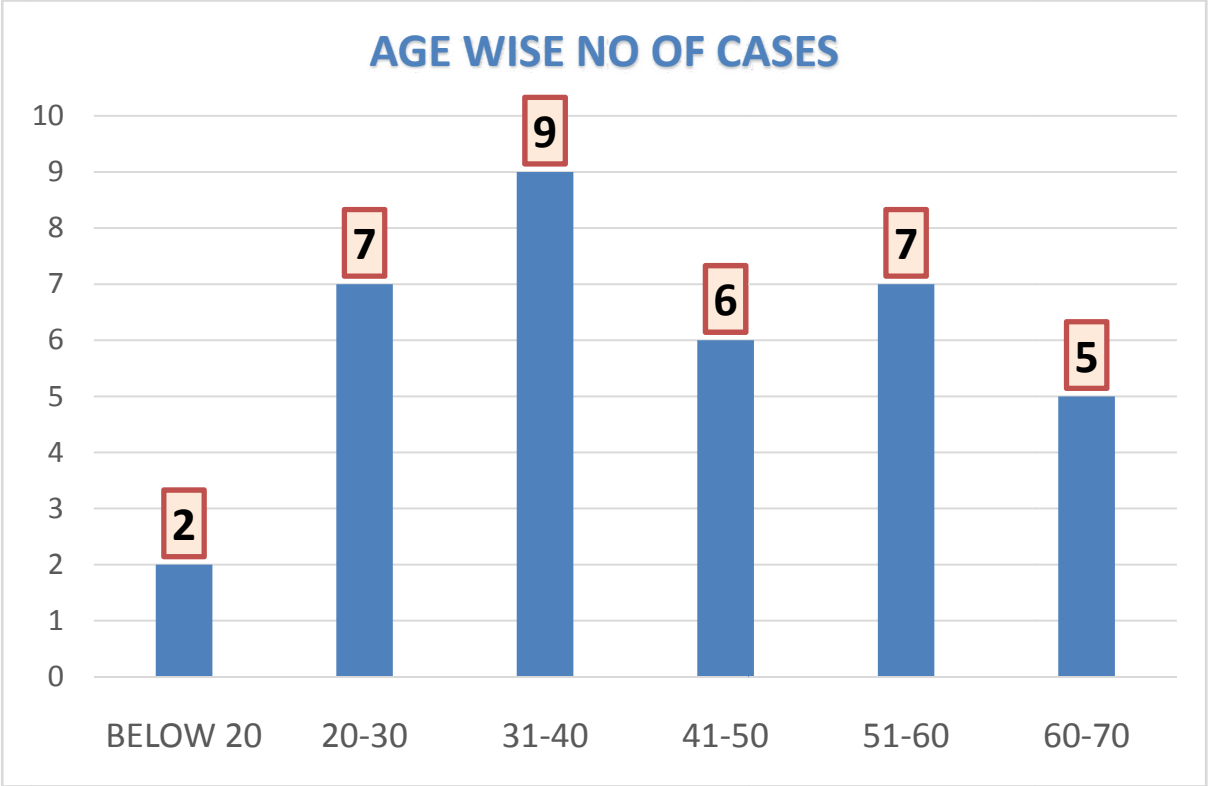
VICERA ANALYSIS REPORT	Count of SL NO
DETECTED OPC	3
NOT DETECTED	5
DETECTED OPC	12
NOT DETECTED	16



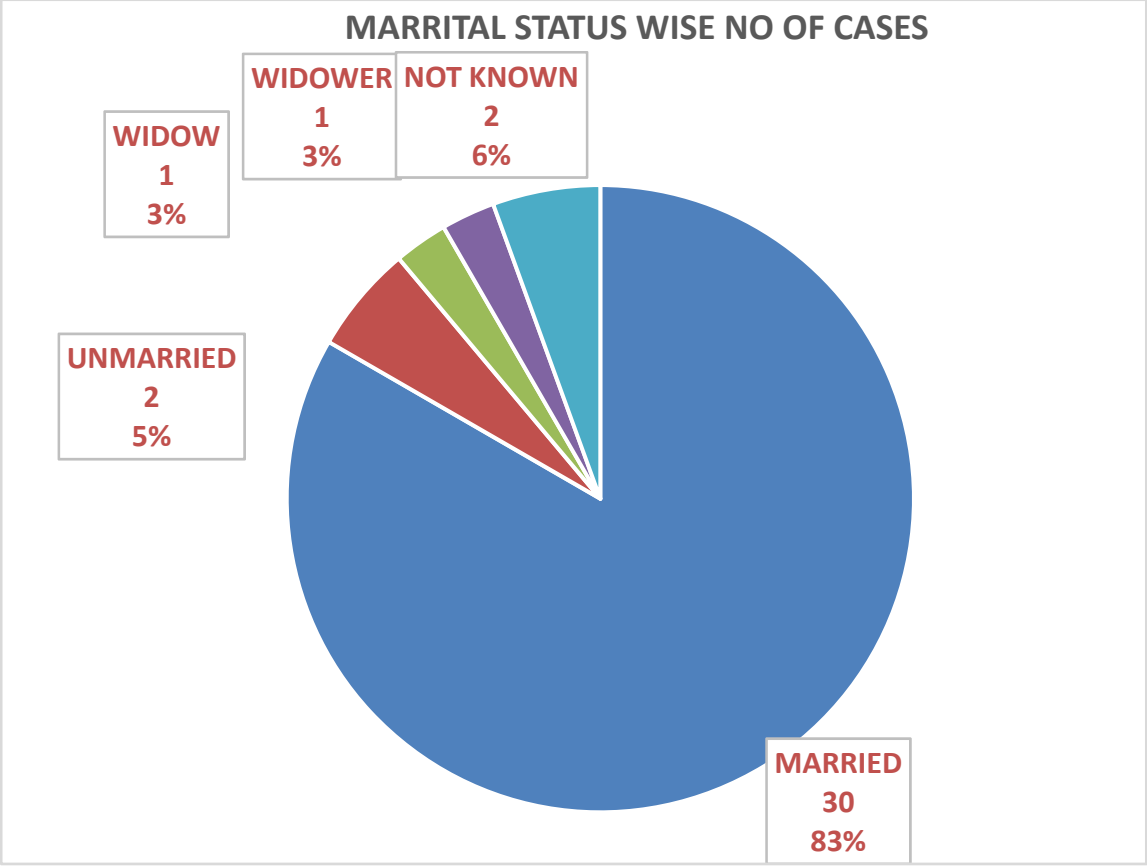
GENDER WISE PM CASES

MALE : 28 CASE

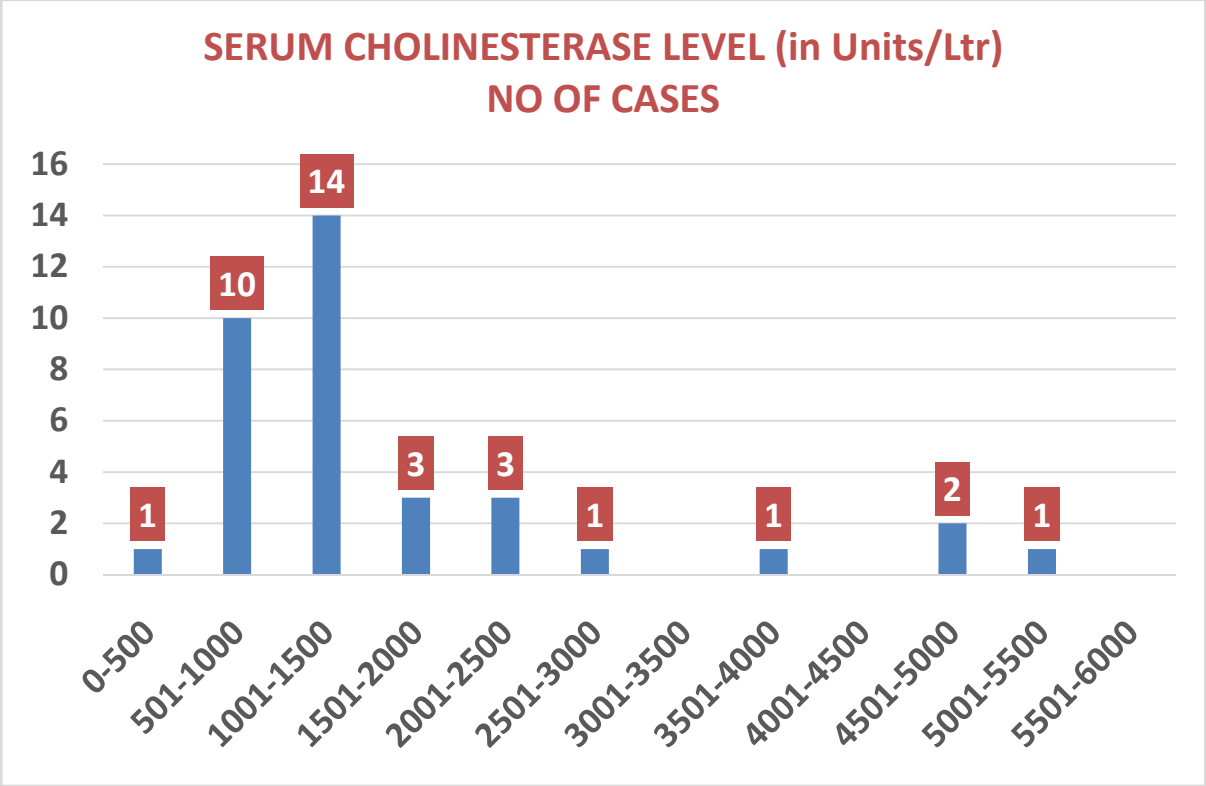
FEMALE : 8 CASES



AGE GROUP IN YEARS	NO OF CASES
BELOW 20	2
20-30	7
31-40	9
41-50	6
51-60	7
60-70	5



MARITAL STATUS	NO OF CASES
MARRIED	30
UNMARRIED	2
WIDOW	1
WIDOWER	1
NOT KNOWN	2



SEERAM COLINESTERACE LEVEL (in Units/Ltr)	NO OF CASES
0-500	1
501-1000	10
1001-1500	14
1501-2000	3
2001-2500	3
2501-3000	1
3001-3500	Nil
3501-4000	1
4001-4500	Nil
4501-5000	2
5001-5500	1
5501-6000	Nil

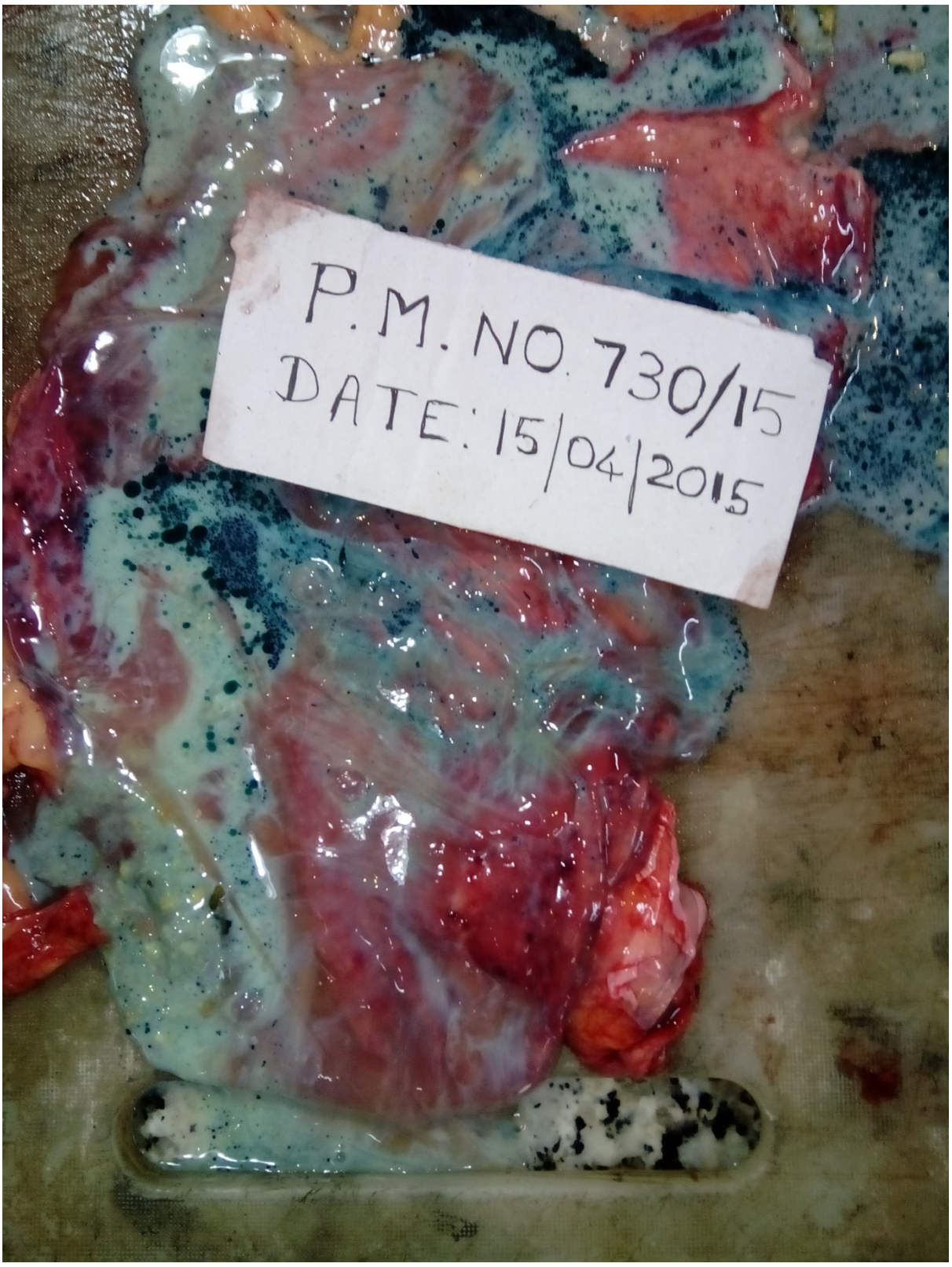
SERUM CHOLINESTERASE LEVEL (in Units/Ltr)

Statistical Analysis Report

Mean	1675.722222
Standard Error	204.7049003
Median	1343.5
Mode	985
Standard Deviation	1228.229402
Sample Variance	1508547.463
Kurtosis	3.266474432
Skewness	1.916457942
Range	5185
Minimum	315
Maximum	5500
Sum	60326
Count	36
Largest(1)	5500
Smallest(1)	315
Confidence Level(95.0%)	415.5730384
P Value	0.05

Photograph of a case of appearance of Stomach

With its contents in OP poisoning



Photograph of a case of appearance of congested lungs with frothy secretions in a case of OP poisoning

7. DISCUSSION

W.H.O. classified pesticides based on the toxic effects as classIa-extremely hazardous to slightly hazardous-Class III. Class Ia pesticides are banned in industrialized countries but used in developing countries.

As different formula of OP are used for poisoning, it is difficult to estimate the dose of the individual formula at which it inhibits the percentage of serum cholinesterase levels . Enzyme inhibition is dependent on the amount absorbed. Various factors are involved in severity of OP poisoning..Baseline values of serum cholinesterase is variable.Serum cholinesterase level varies with age, gender, and other factors.

Though there are controversies in considering serum cholinesterase levels as prognostic indicator, this is accepted as a diagnostic indicator.There was a progressive increase in pseudo-cholinesterase levels in patients treated with Atropine-Pralidoxime in the survived patients. In patients who died and who were also treated with Atropine-Pralidoxime had minimal change from the base line.(saudija.org.) One of the case report had revealed that37years old farmer who consumed parathion OP compound and had cholinesterase level of 140u/l(range3700-11000 u/l), treated with atropine , pralidoxime , developed complications had survived[68] .

Another case was reported who consumed unknown quantity of parathion and became unconsciousand asystole was noticed. He was revived .His cholinesterase level was not detectable.(range3700-11000u/l) He was treated and his course was

complicated by coma.He died on 18th day of admission. His death was attributed to prolonged anoxia[68].

PatientCA farmer aged 63 years, consumed 200ml of parathion several hours earlier,brought in a comatosed condition with bradycardia (32beats/min). He was intubated ,treated with atropine and obidoxime. His serum parathion level was 800micro gram per lit.(toxicabove 10 micro gm per lit.) His serum cholinesterase level was undetectable (range 5400-13,200u/l). He developed complications and was treated. He was discharged to the medical ward on12th day. His cholinesterase level improved to 2000 u/l. As all the signs and symptoms are not present in every patient, and when there is a doubt in the diagnosis,,estimation of plasma cholinesterase or erythrocyteacetyl cholinesterase is valuable. The plasma cholinesterase is used to detect exposure to organophosphorus. The levels of erythrocyte cholinesterase is a good marker of severity of OP poisoning[68] .

In forensic practice this will be a very useful confirmatory test in OP poisoning cases. In my study 36 OP poisoned deceased subjects were studied. Mean value of the serum cholinesterase level among 36 subjects is1675+/-1228.Three subjects were brought dead [68] .

The post mortem findings in subjects who were brought dead had the entire quantity of poison ingested varying from 150ml-800 ml with the specific odour of the insecticide. The stomach mucosa was congested. Lungs were congested. On cut section had the frothy secretions.liver,kidneys,spleen were congested. Brain was also congested.No injuries wereon the body.

in subjects who were treated for 2-3 days had blackish fluid as their stomach contents. Liver, spleen. kidneys and brain,lungs were congested. In subjects treated for more than 3days the post mortem findings were (i) purulent /frothy secretions from the lungs.(ii) Flabby heart with fluid blood.(iii) pale liver,spleenand kidneys and brain.

In subjects who were brought dead the viscera analysis detected OP.Theirserum cholinesterase levels were very low but variable 629u/l, 816u/l and 1131u/l. The presence of the OP in the stomach and other viscera significantly inhibits the serum cholinesterase though variable.

This is similar to the previous studies described by others. In treated subjects the viscera analysis detected OP poison in 12 subjects whose serum cholinesterase levels were as low as 315u/l to 2275u/l. Rest of the subjects whose viscera analysis did not detect OP compounds had their values as low as 985u/l to5500u/l. There are other comorbid conditions like cardiac arrhythmias, A-V block, direct depression of the respiratory centre,Pneumonia and septicaemia leading to the mortality.

In clinical practice this test can done for patients with atypical presentations of symptoms in OP poisoning. In paediatric group from whom it is difficult to elicit history this will be of considerable value. This test is used to assess the health status of the workers in the farm, chemical plant , and farm communities. In Forensic practice for arriving at the cause of death in OP poisoning cases this test will be immense value.Though the higher values do not assist in the diagnosis, lower values are confirmatory.

CONCLUSION

8. CONCLUSION

As pesticides are used for agricultural, domestic ,and industrial purposes, their use cannot be avoided to protect people from starvation, disease ,death, and insect infestations in buildings.(open research online-page2.para1).Debilitating and deadly diseases can be caused by pests, such as insects, rodents, and microbes.So, Environmental Protection Agency has registered pesticide products, and repellants to control the vectors that spread the diseases.

Restricted use pesticides(RUP) is a designation assigned to a pesticide product due to its high degree of human and environmental health hazard on usage according to label instructions.It is available only to the certified pesticide applicators .

Following are the restricted use OP compounds in India: (i)Diazinion.(ii)Fenitrothion.(iii)Fenthion, (iv)Methylparathion and (v)monocrophos

The availability of the OP insecticide is common which renders OP poisoning a worldwide healthproblem,affecting millions of patients with a high fatality rate. The relative unfamiliar compounds in the pesticide makes it difficult for the health care providers to correctly diagnose and treat OP poisoning[67] .

Prophylaxis of poisoning: Due to the serious toxic effects of the pesticides and difficulties in treating the poisoned victims, public should be educated to concentrate on prevention than cure.

Adequate ventilation should be ensured while applying pesticides in homes.

Pesticide label instruction should be strictly followed.

Pesticides should not be transferred to other containers.

Hands should be washed after using pesticides.

Fruits and vegetables should be washed before consumption[12].

As most of the poisoned victims are suicidal,public should be educated about the serious adverse effects of the poisons and miserable death it causes.

National Poison Informationcentre is located at AIIMS institute, New Delhi.It has a computer soft ware on poisons compiled by W.H.O. Regional centres are also located in Chennai and cochin.These centres provide guidelines on toxicity assessment and treatment over telephone.(dr.ksn reddys)

Counselling should be given to the survival victims.

(Persons having suicidal tendency should be takencare.)

REFERENCES

9. BIBLIOGRAPHY

(1) The essentials of Forensic Medicine and Toxicology. By Dr.K.S.Narayan Reddy M.D.,D.C.P.,Ph.D.,F.A.M.S., F.I.M.S.A.,F.A.F.Sc.,F.I.A.M.S.,F.A.F.M. Horonary Professor of Forensic Medicine,S.V.S Medical College,Mahabunagar,(AP)(Retired Principal, Osmania Medical College) and DR.O.P.Murthy M.D., Additional Professor Of Forensic Medicine, All India Institute of Medicinal Sciences, New Delhi Thirty third edition 2014, Section II Toxicology, General considerations(498, 505, 512 , 520)

(2) Text book of Forensic Medicine and Toxicology. Second Edition by Nageshkumar G Rao Bsc, M.B.,B.S.,M.D,F.I.A.M.L.E., F.I.C.F.M.T. Part V: Forensic Toxicology. Laws of poison (522, 526).

(3) The essentials of Forensic Medicine and Toxicology. By Dr.K.S.Narayan Reddy M.D.,D.C.P.,Ph.D.,F.A.M.S., F.I.M.S.A.,F.A.F.Sc.,F.I.A.M.S.,F.A.F.M. (521)

(4) Text book of Forensic Medicine and Toxicology. Second Edition by Nageshkumar G Rao Bsc, M.B.,B.S.,M.D,F.I.A.M.L.E., F.I.C.F.M.T. Part V:Forensic Toxicology. Epidemiology of poisoning, Health effects (422,522,525).

(5) Chest.net.org.dec1994 106/6 1811-14

(6) The essentials of Forensic Medicine and Toxicology. By Dr.K.S.Narayan Reddy M.D.,D.C.P.,Ph.D.,F.A.M.S., F.I.M.S.A.,F.A.F.Sc.,F.I.A.M.S.,F.A.F.M. (124,522)

(7) The essentials of Forensic Medicine and Toxicology. By Dr.K.S.Narayan Reddy M.D.,D.C.P.,Ph.D.,F.A.M.S., F.I.M.S.A.,F.A.F.Sc.,F.I.A.M.S.,F.A.F.M. (523)

(8) The essentials of Forensic Medicine and Toxicology. By Dr.K.S.Narayan Reddy M.D.,D.C.P.,Ph.D.,F.A.M.S., F.I.M.S.A.,F.A.F.Sc.,F.I.A.M.S.,F.A.F.M. (524)

(9) Text book of Forensic Medicine and Toxicology. 1st Edition 2014, By Anil Aggrawal, Director Professor, Dept.of Forensic Medicine and Toxicology, Maulana Azad Medical College, New Delhi. Pat II Toxicology, Section -8 General Principles relating to poisons(544)

(10) Text book of Forensic Medicine and Toxicology. 1st Edition 2014, By AnilAggrawal, Director Professor, Dept. of Forensic Medicine and Toxicology, Maulana Azad Medical College, New Delhi - classification of poisons, Law rerating to Poisons and General Considerations. , Agricultural Poisons (598,599)

(11) Text book of Forensic Medicine and Toxicology. 1st Edition 2014, By AnilAggrawal, Director Professor, Dept. of Forensic Medicine and Toxicology, Maulana Azad Medical College, New Delhi - classification of poisons, Law rerating to Poisons and General Considerations. , Agricultural Poisons (600,601,602,603,604)

(12) Scholars Research Library , Current review on organophosphorus poisoning Page no.199-215.

(13) Text book of Forensic Medicine and Toxicology. Second Edition by Nageshkumar G Rao Bsc, M.B.,B.S.,M.D,F.I.A.M.L.E., F.I.C.F.M.T. Part V:Forensic Toxicology. General Principles (419).

(14) Text book of Forensic Medicine and Toxicology. Second Edition by Nageshkumar G Rao Bsc, M.B.,B.S.,M.D,F.I.A.M.L.E., F.I.C.F.M.T. Part V: Forensic Toxicology. Laws of poison (441,522,526).

(15) Park's Text book of Preventive and social medicinePage no 723 and 724.

(16) Scholar Research Library : S V kumar et al Arch. Appl. Sci. Res., 2010, 2 (4):199-215

(17) Hong Kong Journal of Emergency Medicine, Serial serum Cholinesterase activities as a Prognostic factor in Organophosphate poisoned patients. Page no 92 -97.

(18) Bull,World health org. 1971 ,44, Page No.289-304. Cholinesterase inhibition by organophosphorus compounds and its clinical effects.

(19) The essentials of Forensic Medicine and Toxicology. By Dr.K.S.Narayan Reddy M.D.,D.C.P.,Ph.D.,F.A.M.S., F.I.M.S.A.,F.A.F.Sc.,F.I.A.M.S.,F.A.F.M. (521)

(20) The essentials of Forensic Medicine and Toxicology. By Dr.K.S.Narayan Reddy M.D.,D.C.P.,Ph.D.,F.A.M.S., F.I.M.S.A.,F.A.F.Sc.,F.I.A.M.S.,F.A.F.M. (512)

(21) Clin.chem.31/4, Page no . 546-550.(1985) Total cholinesterase in plasma. Biological variations and reference limits.

(22) J Indian Acad Forensic Med,32(4) Review paper Serum enzymes changes after death and its correlation with time since death.Page No.355-357.

(23) J Nepal med assoc 2008, Correlation of serum cholinesterase level , Clinical score at presentation and severity of organo phosphorus poisoning. Page no.47-52.

(24) Parasuicidalpoisoningby intramuscular injection of insecticide: A case report .online publication July 11, 2013.

(25) Egyptian Journal of Forensic Sciences 2011 pages 93-98.

(26) Journal ofmedical,chemical,biological, and radiological defense.Vol1,2003. Pages 1-9.

(27) Paediatric environmental health speciality unit-2007 1st 2nd pages.

(28) Indian journal of Basic and Applied Medical Research.June2014, Vol-3, Issue-3, Pages285-291.

(29) Journal of medical medical sciences -Turkish pages 279-281)

(30) JABFP,July-Aug1999vol12,n04, pages307-313.

(31) Bio medical vol.24,jul-dec 2008, pages124-129

(32) Thiermann H, Eyer F, Felgenhauer N, Pfab R, Zilker T, Eyer P, et al.Pharmacokinetics of obidoxime in patients poisoned with organophosphoruscompounds.ToxicolLett 2010;197(3):236-42.

(33) Nelson LS, Lewin NA, Howland MA, Hoffman RS, Goldfrank LR, Flomenbaum NE. Goldfrank'stoxicologic emergencies. 9th ed. McGraw Hill; 2011 p.1450-66.

(34) Sam KG, Kondabolu K, Pati D, Kamath A, Pradeep Kumar G, Rao PG. Poisoning severity score, APACHE II and GCS: effective clinical indices for estimating severity and predicting outcome of acute organophosphorus and carbamate poisoning. J Forensic Leg Med 2009;16(5):2 39-47.

(35) Kang EJ, Seok SJ, Lee KH, Gil HW, Yang JO, Lee EY, et al. Factors for determining survival in acute organophosphate poisoning. Korean J Intern Med 2009; 24(4):362-7.

(36) Chuang FR,JangSW,Lin JL, ChernMS,Chen JB, Hsu KT.QTc prolongation indicates a poor prognosis in patients with organophosphate poisoning.AmJEmerg Med 1996;14(5):451-3.

(37) Aygun D, Doganay Z, Altintop L, Guven H, Onar M, Deniz T, et al. Serum acetylcholinesterase and prognosis of acute organophosphate poisoning. J ToxicolClinToxicol 2002;40(7):903-10.

(38) Akdur O, Durukan P, Ozkan S, Avsarogullari L, Vardar A, Kavalci C, et al. Poisoning severity score, Glasgow coma scale, corrected QT interval in acute organophosphate poisoning. Hum ExpToxicol 2010; 29(5):419-25.

(39) Robey WC III, Meggs WJ. Pesticides. In Tintinalli'semegency medicine: a comprehensive study guide. 7th ed. McGraw-Hill and ACEP; 2011. p.1297-305.

(40) Nouira S, Abroug F, Elatrous S, Boujdaria R, Bouchoucha S. Prognostic value of serum cholinesterase in organophosphate poisoning. Chest 1994;106(6): 1811-4.

(41) MerillDG,MihmFG. Prolonged toxicity of organophosphate poisoning. Crit Care Med 1982;10 (8):550-1.

(42) Routier RJ, Lipman J, Brown K. Difficulty in weaning from respiratory support in a patient with the intermediate syndrome of organophosphate poisoning. Crit Care Med 1989;17(10):1075-6.

(43) Hassan RM, Pesce AJ, Sheng P, Hanenson IB. Correlation of serum pseudocholinesterase and clinical course in two patients poisoned with organophosphate insecticides. ClinToxicol 1981;18(4):401-6.

(44) Brahmi N, Mokline A, Kouraichi N, Ghorbel H, Blel Y, Thabet H, et al. Prognostic value of human erythrocyte acetylcholinesterase in acute organophosphate poisoning. Am J Emerg Med 2006;24(7):822-7.

(45) Kolf-Clauw M, Jez S, Ponsart C, Delamanche IS. Acetyl- and pseudo-cholinesterase activities of plasma, erythrocytes, and whole blood in male beagle dogs using ellman's assay. Vet Hum Toxicol 2000;42(4):216-9.

(46) Sakr Y, Madl C, Filipescu D, Moreno R, Groeneveld J, Artigas A, et al. Obesity is associated with increased morbidity but not mortality in critically ill patients. Intensive Care Med 2008;34(11):1999-2009.

(47) Orluwene CG, EjilemeleAA. Comparison of red cell cholinesterase and plasma cholinesterase activities in early detection of organo-phosphorus

toxicity in exposed industrial workers in Port Harcourt, Nigeria.Niger J Med 2006;15(3):314-7.

(48) Chen HY, Wang WW, Chaou CH, Lin CC. Prognostic value of serial serum cholinesterase activities in organophosphate poisoned patients. Am J Emerg Med 2009;27(9):1034-9.

(49) Eddleston M, Eyer P, Worek F, Sheriff MH, Buckley NA. Predicting outcome using butyrylcholinesterase activity in organophosphorous pesticide self-poisoning. QJM 2008;101(6):467-74

(50) Arnason A., Bjarnason O. (1972). Postmortem changes of human serum esterasesActaPatholMicrobiolScand (A) 80; 841 - 846.

(51) Biswas G. (2010). Review of Forensic Medicine & Toxicology, Jaypee Publication, 1 st edition; 109.

(52) Coe J.I. (1974). Postmortem chemistry, practical considerations and a review of literature J. Forensic Sci; 19:13 - 32.

(53) Cox H.M.V., Sinha U.S., Knight B. (1995). Medical Jurisprudence& Toxicology 6 th edition The law book company (P) ltd. Allahabad:206.

(54) Dixit P.C. (2007). Text Book of Forensic Medicine & Toxicology, Peepe Publishers 1st edition: 110.

(55) Enticknap J.B. (1960). Biochemical changes in cadaver sera J. Forensic Med; 7:135 - 146.

(56) Evans WED (1963). The Chemistry of Death Charles C. Thomas Springfield; 100.

(57) Garg S.P., Arora A, Dubey BP (2005). A study of Serum Enzymal changes after Death & its correlation with Time since Death.

(58) JIAFM: 27 (1). ISSN 0971 - 0973

(59) Glaister (1973).Medical Jurisprudence & Toxicology 13 th edition Churchil Livingston: 118.

(60) Gradohl. Legal medicine (1976). 3rd edition (Indian) K M Verghese Company Bombay: 95 - 97.

(61) Hall W.E.B. (1958). The medicolegal application of the serum transaminase test J. Forensic Sci; 3:117 - 122.

(62) Knight B. Lawyer□s guide to Forensic Medicine William Heinmann Medicine Books Limited, London 1992.

(63) Knight B, Henssge C, Kranpecher T, Madea b, Nokes L. The estimation of time since death in the early postmortem period Arnold Publishers 1995

(64) Lythgoe A.S. (1980). T he activity of Lactate Dehydrogenase in cadaver sera: a comparison of different sampling sites. Med. Sci. Law Vol. 20, No. 1: 48 - 53.

(65) Mukherjee J.B. (1994). Forensic Medicine& Toxicology 2 nd edition Arnold Associates Vol. I ; 253 - 254.

(66) Modi JP and Subramani um B. V. (1998). Medical Jurisprudence& Toxicology. 22nd edition, Butterworths Publication: 246.

(67) Nandy A. (2010). Principles of Forensic Medicine including Toxicology 3 rd edition: 280.

(68) Naumann H.N. Postmortem (1956). Liver Function Tests. Am. J. Clin. Pat hol. 26: 495 - 505.

(69) Parikh C. K. Textbook of Medical Jurisprudence & Toxicology (1992). 5 th edition Medico - legal Center, Bombay 173

(70) Petty C.S., Lovel M.P., Moore F. (1958). Organophosphorus insecticides and postmortem blood cholinesterase levels J. Forensic Sci 3 226 - 237.

(71) Fromhttp://www.toxicologyinternational.com Sep22,2012. Page no255-259.

(72) Evtugyn GA, Budnikov HC, Nikolskaya EB. Sensitivity and selectivity of electrochemical enzyme sensors for inhibitor determination. Talanta. 1998;46:465–84. [PubMed]

(73) Fukuto TR. Mechanism of action of organophosphorus and carbamate insecticides. Environ Health Perspect. 1990;87:245–54. [PMC free article] [PubMed]

(74) Bajgar J. Organophosphates/nerve agent poisoning: Mechanism of action, diagnosis, prophylaxis, and treatment. AdvClin Chem. 2004;38:151–216. [PubMed]

(75) Ganesan K, Raza SK, Vijayaraghavan R. Chemical warfare agents. J Pharm Bioallied Sci. 2010;2:166–78. [PMC free article] [PubMed]

(76) Hasin Y, Avidan N, Bercovich D, Korczyn AD, Silman I, Beckmann JS, et al. Analysis of genetic polymorphisms in acetylcholinesterase as reflected in different populations. Curr Alzheimer Res. 2005;2:207–18. [PubMed]

(77) Dyer SM, Cattani M, Pisaniello DL, Williams FM, Edwards JW. Peripheral cholinesterase inhibition by occupational chlorpyrifos exposure in Australian termiticide applicators. Toxicology. 2001;169:177–85. [PubMed]

(78) Senanayake N, de Silva HJ, Karalliedde L. A scale to assess severity in organophosphorus intoxication: POP scale. Hum ExpToxicol. 1993;12:297–9. [PubMed]

(79) Taylor P. Anticholinesterase agents. In: Gilman AG, Goodman LS, editors. The pharmacological basis of therapeutics. New York: Macmillan Publishing Co. Inc; 1985. pp. 110–28.

(80) Benmoyal-Segal L, Vander T, Shifman S, Bryk B, Ebstein RP, Marcus EL, et al. Acetylcholinesterase/paraoxonase interactions increase the risk of insecticide-induced Parkinson's disease. FASEB J. 2005;19:452–4. [PubMed]

(81) Ellenhorn MJ. Pesticides. In: Ellenhorn M.J, editor. Ellenhorn's Medical Toxicology Diagnosis and Treatment of Human Poisoning. second ed. Baltimore, USA: William and Wilkins; 1997. pp. 1614–63.

(82) Abdullat IM, Battah AH, Hadidi KA. The use of serial measurement of plasma cholinesterase in the management of acute poisoning with

organophosphates and carbamates. Forensic Sci Int. 2006;162:126–30. [PubMed]

(83) Zhang X, Li HS, Zhu QH, et al. Trends in suicide by poisoning in China 2000–2006: age, gender, method, and geography. Biomed Environ Sci.2008;21:253–256. [PubMed]

(84) Buchman MT. Upper extremity injection of household insecticide: a report of five cases. J Hand Surg Am. 2000;25:764–767. [PubMed]

(85) Fratello U, D'Auria C, De Vita A, Fasano C, Marra F, et al. Unusual case of acute organophosphate ester poisoning by parenteral way. Minerva Anestesiol. 1974;40:331–336. (In Italian) [PubMed]

(86) Güven M, Unlühizarci K, Göktaş Z, Kurtoğlu S. Intravenous organophosphate injection: an unusual way of intoxication. Hum ExpToxicol. 1997;16:279–280. [PubMed]

(87) Worek F, Koller M, Thiermann H, Szinicz L. Diagnostic aspects of organophosphate poisoning. Toxicology. 2005;214:182–189. [PubMed]

(88) Paudyal BP. Organophosphorus poisoning. JNMA J Nepal Med Assoc. 2008;47:251–258. [PubMed]

(89) Jokanović M. Structure-activity relationship and efficacy of pyridiniumoximes in the treatment of poisoning with organophosphorus compounds:a review of recent data. Curr Top Med Chem. 2012;12:1775–1789. [PubMed]

(90) Güloglu C, Aldemir M, Orak M, Kara IH. Dichlorvos poisoning after intramuscular injection. Am J Emerg Med. 2004;22:328–330. [PubMed]

(91) Yucel I, Demiraran Y, Alper M. Suicide attempt with injection of insecticide in both wrists. Orthopedics. 2008;31:174. [PubMed]

(92) Bala I, Pratap M, Nakra D, Ramprabhu T. Prolonged cholinergic crisis and compartment syndrome following subcutaneous injection of an organophosphate compound for suicide attempt. J Forensic Leg Med. 2008;15:256–258. [PubMed]

(93) Aydin A, Aköz F, Erer M. Subcutaneous injection of insecticide for attempted suicide: a report of two cases. ActaOrthopTraumatolTurc. 2004;38:295–297. (In Turkish) [PubMed]

(94) Chest journal article Dec 1994 , Prognostic value of Serum cholinesterase in OP poisoning.

(95) Indian Medical gazette Jan 2014 Lab abnormalities in Patients with organo phosphorus poisoning.

(96) International journal of medical research and review. Study of serum cholinesterase , CPK, LDH prognostic biomarkers in OP poisoning.

(97) Asia pacific journal of medical Toxicology. Article7,volume2, issue,1, march 2013, Pages23-27.

(98) International journal of recent trendsin science and technology.Volume 9, Issue2, pages 270-274.

(99) International journal of pharma and biosciences iss 975, pages 785, 786.